Predicting Phosphorus Limitations in *Eucalyptus* Plantations

by

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Declaration

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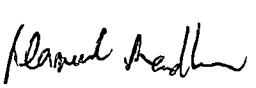

Daniel Steven Mendham

Abstract

Phosphorus (P) fertilizers are currently used during the establishment phase of most eucalypt plantations. Phosphorus fertilizer is applied at a similar rate to nearly all sites, but eucalypts respond to P only at some sites. Site-specific management would improve the efficacy of P fertilizer use, so soil indicators of P fertilizer response in new eucalypt plantations warranted evaluation.

Research focussed on soil solution P, because that is the pool of P directly available to plant roots. Concentration of P in solution (both bulk soil and that predicted at the root surface) was related to rates of uptake and growth in solution- and soil-based studies. These and more commonly used indicators of soil P status were calibrated and compared in field experiments.

The phosphate uptake characteristics of *Eucalyptus nitens* roots were characterised. The high affinity uptake system had a Michaelis constant (K_m) of 0.37 µM P in solution, and a maximal uptake rate (I_{max}) of 43 nmol/g/hour (fresh weight basis). Concentrations of 0.19-0.58 µM P in solution, and 0.20 µM $CaCl_2$ P were required for 90% of maximum growth in pot experiments with four soils of contrasting P buffer power. These experiments indicated that *E. nitens* roots had a high affinity for P, and that optimal growth in soil occurred at a similar concentration to the K_m value. The relationship between Colwell extractable P and growth was well correlated within each soil type, but unlike P indicators of intensity (solution P and $CaCl_2$ P), the relationship was not common to different soil types.

An excellent relationship ($R^2 = 0.81$) was found between $CaCl_2$ extractable P (range: 0.1-0.5 µM) and growth response at 1 year of age to P applied at planting in 21 field experiments in Tasmania, Victoria, New South Wales, and Western Australia (90% of maximum growth occurred at 0.50 µM). Three sites from Victoria did not fit this trend. Quantity-based P analyses, such as Bicarbonate P (range: 2 - 63 µg/g), Bray No. 2 P (0.1 - 15 µg/g) and Acid extractable P (0.6 - 11 µg/g) were less well correlated with response to P fertilization at

planting (R^2 = 0.48, 0.43, and 0.35, respectively).

The concentration of P at the root surface was predicted using models which integrated the theory of P supply and uptake. The concentration of P at the root surface was assessed for its ability to predict P deficiency in maize using growth responses to P fertilization in a published pot experiment with 26 Queensland soils. Predicted concentration at the root surface was well correlated with P uptake (R^2 = 0.95) and response to P fertilizer (R^2 = 0.93). Glasshouse experiments were used to examine the relationship between predicted concentration at the root surface and response to P fertilizer in *E. nitens* and *E. globulus*. Good correlations were also found for these species between predicted concentration of P at the root surface and response to P fertilizer (R^2 = 0.63 - 0.99) within each soil type, but the relationships were highly specific to soil type.

In conclusion, an indicator of P intensity ($CaCl_2$ P) was well correlated with response to P fertilizer in young eucalypt plantations, and a common relationship was found in a range of soil types. $CaCl_2$ P was more highly correlated with response in different soil types than both quantity-based indicators (Bicarbonate, Bray no. 2, Acid extractable P), and predicted concentration of P at the root surface.

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I also wish to thank my family, including Neville, Robyn, Nicholas, Jared and Bonita Mendham, who have been sources of inspiration and encouragement. I especially wish to thank my wife, Tara, for her encouragement and support.

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List of Symbols

Symbol	Definition	Typical units
ρ	Soil bulk density	g/cm^3
η	Water viscosity	-
θ_v	Volumetric water content	cm^3 water/cm^3 soil
b	Soil P buffer power	-
C_l	Concentration of P in the liquid phase	µM or mM
C_{la}	Concentration of P at root surface	µM or mM
C_{min}	Concentration where net influx is zero	µM or mM
C_s	Concentration of P sorbed to solid phase	mol/cm^3
C_t	Total diffusible P in soil	mol/cm^3
D_e	Effective diffusion coefficient	cm^2/sec
D_l	Diffusion coefficient in liquid	cm^2/sec
f	Impedance factor	-
I	Rate of influx into roots	nmol/g FW/hour
I_{max}	Maximal rate of influx	nmol/g FW/hour
k_B	Boltzmann constant	$g.cm^2/s$
K_d	Solid-liquid partition coefficient	mL/g
K_m	Michaelis constant	µM or mM
n	Tissue N concentration	g N/g DW
N_s	Concentration of N in solution	µM
p	Tissue P concentration	g P/g DW
P_i	Inorganic orthophosphate	-

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Table of Symbols continued...

Symbol	Definition	Typical units
P_s	Concentration of P in solution	μM or mM
P_x	Phosphorus treatment level x	-
r	Root:Shoot ratio	-
R_0	Root radius	cm
R_D	Depletion zone radius	cm
RAR	Relative addition rate	/day
RGR	Relative growth rate	/day
r_i	Ionic radius	cm
R_S	Weight of root fragments in subsample	g
R_T	Total weight of root fragments	g
R_x	Relative addition rate of nutrient x	/day
SAR	Specific absorption rate	(g/g) /day
S_S	Weight of soil+root subsample	g
S_T	Total weight of soil+roots	g
t	Time	d
T	Absolute temperature	K
U	Uptake rate	nmol/g FW/hour
v	Water influx to the root	$cm^3/cm^2/s$
W_p	Weight of phosphorus	g
W_r	Weight of roots	g

Note: If a symbol is used for a different meaning, or has different units, it is noted in the text.

1. Introduction

The need to increase hardwood fibre production and public pressure to reduce harvesting of native forests has encouraged forest industries in Australia to turn to eucalypt plantations for high quality pulp and paper resources, as well as solid wood products (eg. Helsham *et al.* 1988). Fertilization is one management option that can increase growth rates (Schönau and Herbert 1989), shorten rotation lengths, and hence improve the economic viability of eucalypt plantations in Australia (Gerrand *et al.* 1993).

Eucalypt plantations in Australia are currently being established at the rate in excess of 14 000 ha/year (Cromer 1996, 1993 estimate) on sites with a wide range of soils and previous land uses, including pasture, *Pinus* sp. plantations, native eucalypt forest, and rainforest (Skinner and Attiwill 1981, Wang *et al.* 1996a). *Eucalyptus nitens* and *E. globulus* are the main species currently planted, occupying approximately 80% of the area of temperate hardwood plantations and 62% of the total area of hardwood plantations in Australia (National Forest Inventory 1997). Despite establishment of large areas of *E. nitens* and *E. globulus* plantations, there have been few detailed investigations into P uptake characteristics of eucalypt roots, or into relationships between soil tests likely to be useful indicators of P deficiency in these plantations.

Improved understanding of P supply and uptake relations in soils would enable more efficient management of P on a site-specific basis, thereby saving fertilizer on sites that do not require P, and increasing growth at sites that require more P than is currently applied. Improved P fertilizer management would also reduce potential environmental damage caused by leaching of excess phosphate fertilizer into streams and groundwater.

Significant, sustained growth responses to P fertilization have been obtained in Australia (Cromer and Williams 1982, Ward *et al.* 1985, Cromer *et al.* 1993, Judd *et al.* 1996a, Bennett *et al.* 1997), and P fertilizer is routinely applied to new plantations (Cromer 1996). The

magnitude of the response to P fertilization can vary both between soil types (Bennett *et al.* 1997) and within a single soil type (G. K. Holz, pers. comm.), but few fertilization régimes differentiate between responsive and non-responsive sites. One company in Victoria has developed fertilizer prescriptions for *Pinus radiata* plantations based on soil type (Turvey 1980). In that study, 80 000 ha of Australian Paper's estate were classified into 31 map units, and grouped into 3 corresponding management classes for *Pinus radiata*. Other forestry organizations in New South Wales, Queensland and Tasmania use soil maps to stratify their plantation resource (Birk 1994), mainly for managing *Pinus radiata*. Although soil-type stratification exists, the knowledge base for site specific management is very limited. Currently there are no guidelines for site specific fertilization regimes in temperate eucalypts, and there are no fertilization regimes in eucalypts that are based on soil-analyses.

Much research has been conducted into managing P fertilizers in agricultural crops (eg. Holford 1997) and *Pinus* plantations (eg. Birk 1994). Agricultural P analyses have proven to be useful for predicting response to P fertilization in *Pinus* plantations (eg. Ballard and Pritchett 1975), but such tests are generally soil- and crop-type specific (Bolland *et al.* 1994, Holford 1991, 1997). Analyses that are useful for agricultural crops and *Pinus* plantations need further evaluation for eucalypts, because different plant species have different requirements for P (Asher and Loneragan 1967) and utilize different pools of soil P (Holford 1991).

A number of P fertilizer field experiments have been established with *E. nitens* and *E. globulus* on a broad range of sites in southern Australia (eg. Judd *et al.* 1996a, Holz 1997). Such experiments predominantly compare rates of P required to obtain optimal growth on specific soil types, but there has been no attempt to develop a soil-based indicator of P deficiency. The opportunity existed to use these field experiments, in conjunction with pot experiments, to examine relationships between pools of soil P, soil P analyses, and growth response over a wide range of sites and soil-types.

Current knowledge of processes affecting P supply and uptake allows prediction of the concentration of a nutrient that develops at a root surface, and consequently uptake by plants (Nye and Tinker 1977, Barber 1984). This approach offers an opportunity to identify P-deficient sites, based on specific soil and plant parameters. Such an approach may be less specific to soil- and plant-type than current indices of P availability, and thereby applicable to the wide range of sites currently being planted to eucalypts.

2. Literature review

2.1 Scope of this review

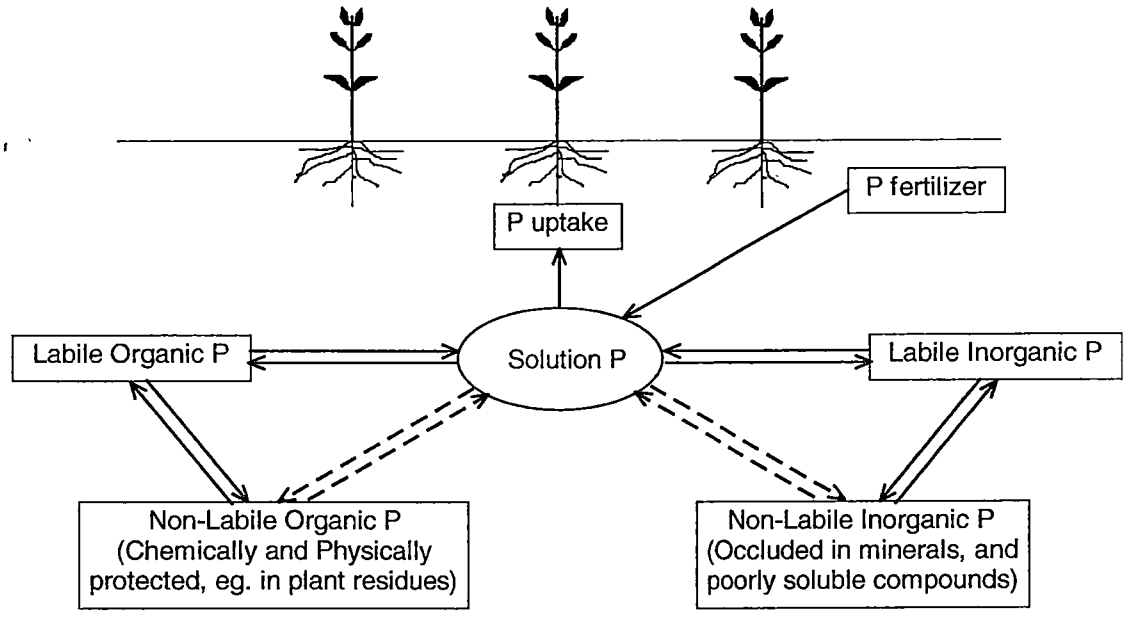
The purpose of this review was to identify gaps in current knowledge that limit the use of indicators of soil phosphorus availability in eucalypt plantations. Phosphorus dynamics in the soil-plant system are reviewed, and implications for choice of P analysis and fertilizer management are discussed. The main processes affecting nutrient acquisition by plants are availability in the soil, and the ability of the plant to take up those nutrients. Both processes are reviewed separately, and then models that integrate soil supply and plant uptake are discussed. The range and limitations of current soil P analyses are reviewed, and their potential application to eucalypt plantations is discussed. The hypothesis is developed that the concentration of P predicted at the root surface may be a useful indicator of sites that require P fertilizer to increase growth.

2.2 Soil phosphorus availability

2.2.1 Pools of soil P

Organic and inorganic forms of phosphorus in soil are present in both the liquid and solid phases (Figure 2.1), but inorganic orthophosphate in soil solution (P_i) is the only form of P that readily crosses the plasma membrane of plant roots (Marschner 1995). Some uptake of other forms of P occurs at the root tip, prior to formation of the casparian band, but uptake via the plasma membrane of root cells is the source of most P taken up by plants (see Section 2.3)

Figure 2.1 - Diagrammatic representation of the P cycle in soil (after Tisdale *et al.* 1993)



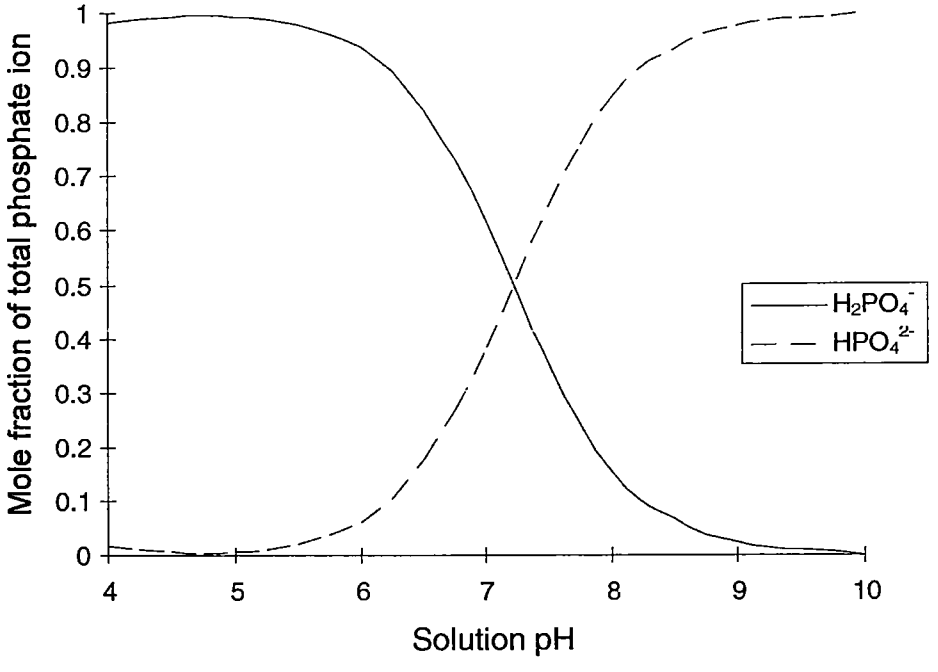
Solution P is a very minor component of the total quantity of P in most soils. For example, data from Dalal and Hallsworth (1976) showed that calcium chloride extractable P [which Moody *et al.* (1988) found was highly correlated with solution P] accounted for between 0.000142 and 0.015 percent of the total P in 20 Australian soils with a wide range of P characteristics (calculated using an assumed soil water content of 30%).

The quantity of P present in the soil solution pool at any one time is depleted many times over during the course of a growing season, and hence must be replenished from other pools of soil P. Short term replenishment is mainly from the labile phase, while replenishment of the labile pool from other fractions of the solid phase occurs over longer time scales (Barrow 1989, 1992).

The form of the phosphate ion in solution is dependent on the pH of the soil solution. The $H_2PO_4^-$ ion is prevalent between pH 2 and 7, and the HPO_4^{2-} ion is more prevalent between pH 7 and 12 (Figure 2.2). The $H_2PO_4^-$ ion is the main form of P transported across the plasma-membrane (Clarkson and Grignon 1991, see Section 2.3.5), so uptake of P mainly

occurs at lower pH's in solution. However, solution pH is not directly related to uptake, because the pH at sites of P uptake (ie. the plasma membrane) is partially buffered by the plant (see Section 2.3.5).

Figure 2.2 - Ionic form of phosphate ion over the range of solution pH normally encountered by plants (after Barber 1984)



While it is useful to discuss the various pools of labile and non-labile P in soil, divisions between the pools are not well defined in soil. There is more likely to be a continuum of chemical states of P, due to heterogeneity of soil components (Fixen and Grove 1990). Non-labile inorganic P is comprised of P in primary and secondary minerals (Tisdale *et al.* 1993), and also P occluded in clay and iron (Fe) and aluminium (Al) oxide and hydroxide minerals (Barrow 1989). The term *phosphate sorption* is used to describe the chemical reactions between phosphate ions and soil minerals. In acidic soils, minerals predominantly involved in sorption are Fe and Al oxides and hydroxides. In alkaline soils, precipitation reactions with calcium are more prevalent (Stevenson 1986), but sorption still occurs to Fe and Al hydroxides. Sorbed P is the main form of labile and some of the non-labile inorganic P in

acid soils.

Organic P can comprise a significant component of total soil P, for example, Kelly *et al.* (1983) found that organic P accounted for 26 to 81% of total soil P in a range of forest soils from northern NSW. Large proportions of organic P in forest soils have led researchers to investigate organic P and biogeochemical cycling (Switzer and Nelson 1972) as indicators of site quality in forests, and to explain the natural distribution of eucalypts in Australia (Adams 1996). Clearing and burning (Romanya *et al.* 1994), or just burning (Chambers and Attiwill 1994) cause rapid loss or mineralization of much of the organically bound P in the soil. The ratio of organic P to inorganic P in a *Eucalyptus diversicolor* succession has been shown to increase over time (Adams 1992). Hence inorganic P may be more important during plantation establishment than later in the rotation.

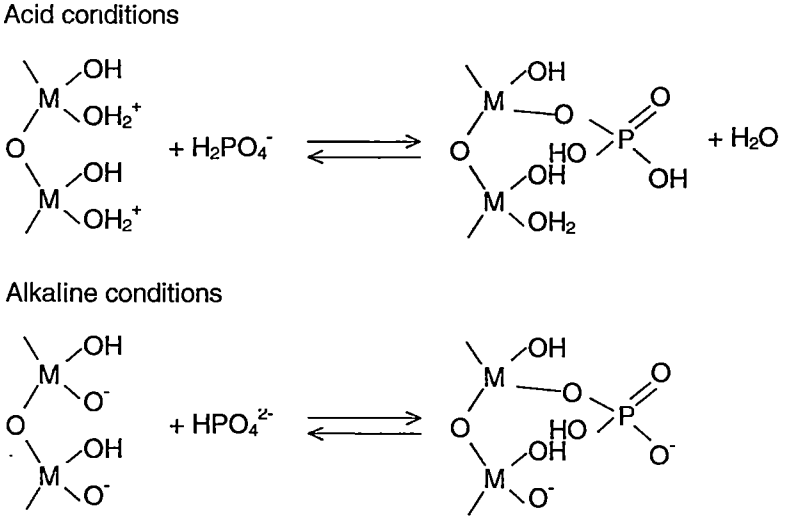
2.2.2 P sorption in soils

The phenomenon of sorption is mainly the reason why solution P accounts for only a small fraction of available soil P. Experimentally, it is difficult to tell the difference between adsorption/desorption and precipitation/dissolution reactions (Veith and Sposito 1977), because both of these processes affect the concentration in soil solution. Precipitation with calcium is the main effect at high pH, while sorption to the Fe and Al oxide and hydroxide minerals primarily occurs at low pH. Sorption to variable charge clay surfaces also contributes to sorption at low pH.

Clay content is approximately correlated with P sorption capacity, but sorption is related more to the quantity of variable charge than the amount of clay *per se*. Ballard and Fiskell (1974) found that sorption was better correlated with the quantity of amorphous iron and aluminium than with clay, and Singh and Gilkes (1991) found linear correlation coefficients of 0.6 to 0.8 between the Freundlich 'a' sorption coefficient and extractable iron and aluminium, and only 0.32 between the Freundlich 'a' sorption coefficient and clay content of 97 soils from south-western Australia.

Organic matter also influences the sorption characteristics of a soil. Ballard and Fiskell (1974) found a highly significant positive correlation between loss on ignition and sorption capacity, and Saunders (1965) found a positive relationship between organic carbon and P retention. The correlations were probably due to Al and Fe adsorbed by organic colloids (Tisdale *et al.* 1993). Where organic matter is added to soil, P sorption can be reduced via competition of conjugate anions (from organic acids produced during organic matter breakdown) for P sorption sites (Iyamuremye *et al.* 1996), but the effect is only temporary (Afif *et al.* 1995).

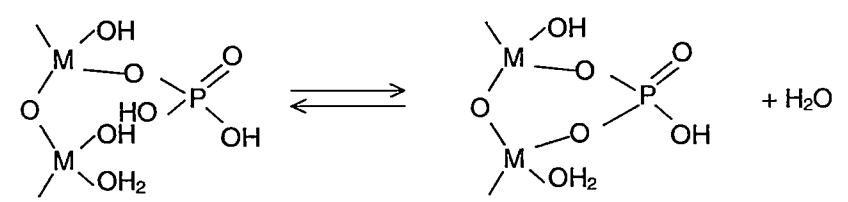
Figure 2.3 - A mechanism for sorption of P to aluminium or iron (represented by M) oxides (after Mott 1981)



Phosphate sorption in acid soils occurs mainly via ligand exchange with the Fe and Al oxide and hydroxide minerals. Positive charges on the Fe and Al hydroxide minerals at low pH attract the $H_2PO_4^-$ ion, which exchanges with H in the OH_2^+ groups, releasing a water molecule (Figure 2.3). In alkaline soils, the HPO_4^{2-} ion is electrostatically repelled from the OH^- groups on the minerals, but a high chemical affinity exists for the surface, and a reaction similar to that shown in Figure 2.3 can occur (Barrow 1989). These reactions are readily reversible, and are the source of readily labile P.

The monodentate reaction product of the initial sorption reaction is thought to undergo further ligand exchange over time to form a more stable bi-nuclear complex (Mott 1981, Figure 2.4). The bi-nuclear complex was thought to exhibit non-reversible behaviour (Mott 1981), but Barrow (1983b) showed that sorption was fully reversible and he proposed a different mechanism to explain the slow sorption phenomenon (eg. Barrow 1983a, 1987 and 1989). Barrow hypothesised that initial adsorption (Figure 2.3) was followed by diffusive penetration into the Fe and Al minerals over time, such that there was a continuum of P availability, from that readily available at the surface to that deep inside the layers of the P sorbing minerals. Phosphate deep inside the minerals may take years to diffuse to the surface and become available to plants. The diffusive penetration theory of Barrow was supported by the observation of Scheidegger and Sparks (1996) that organic chemicals (which also exhibit slow sorption) can be attacked by soil microbes if they are in the labile phase in the soil, but are protected from microbial attack if they are in the non-labile, or slowly sorbed phase.

Figure 2.4 - Formation of binuclear stable complex (after Mott 1981)



Experimentally, phosphate sorption curves have been extensively used to describe the relationship between the solid and liquid phases of P (e.g. Fox & Kamprath 1970, Holford & Mattingly 1976, Saunders 1965, Singh & Gilkes 1991). Sorption curves are produced either by addition of different levels of P to the soil (adsorption curves), or by depletion of phosphorus with successive extractions or with different solution:soil ratios (desorption curves). A sorption curve is a plot of the quantity of P adsorbed or desorbed versus the concentration remaining in solution. The standard time of extraction for an adsorption curve is 17 hours (Rayment and Higginson 1992), but full equilibrium is not reached, even after the

equivalent of 1000 days at 25°C, due to the nature of the slow sorption reaction (Barrow and Shaw 1975). The standard 17 hour extraction provides a measure of the short-term sorption ability of the soil, but it cannot be used to predict long-term changes in soil P status.

Several mathematical functions can describe P sorption curves (eg. Sibbesen 1981), but two that are frequently used are the Langmuir ($y = \frac{abx}{1+ax}$) and Freundlich ($y = ax^{1/b}$) functions, where y is the amount of P sorbed (e.g. µg P/g soil), x is the concentration remaining in solution (e.g. µg P/mL), and a and b are fitted values. The Langmuir function was developed to describe concentration dependent sorption of molecules onto surfaces (at a constant temperature). The parameters of the Langmuir function (ie. a and b) describe useful attributes of sorption in surface chemistry; the a coefficient is an affinity parameter, while the b coefficient is the adsorption maximum (ie. when the surface is saturated). The Langmuir function is preferred by some researchers for description of P sorption by soil, because of its theoretical basis and close fit to some observations (eg. Olsen and Watanabe 1957, Holford 1997). However, the Langmuir function poorly describes sorption of phosphate in the majority of soils (Barrow 1978), indicating that simple surface chemistry is usually a poor analogue of P sorption in soils. The Freundlich function does not reach a plateau at higher P concentrations, and describes sorption better in most soils.

As an example of the relative effectiveness of the Freundlich and Langmuir functions for describing P sorption curves, soils from two published P sorption studies (Ballard and Fiskell 1974, and Fox and Kamprath 1970) were reanalysed to obtain Freundlich and Langmuir fits (Table 2.1).

Table 2.1 - Sorption parameters of selected soils from published experiments, in order of increasing sorption capacity[A].

Soil Type	Freundlich coefficient a	Freundlich coefficient b	Langmuir coefficient a	Langmuir coefficient b	Reference[B]
St Johns sand	0.32	0.31	-	-	a
Leon sand	0.35	0.95	4.00×10^{-3}	108.86	b
Chipley sand	72.14	3.04	0.70	177.21	a
Norfolk sand	112.96	2.89	2.97	176.76	b
Portsmouth sandy loam	145.66	2.44	2.02	246.11	b
Rutledge sand	145.72	3.24	0.75	337.22	a
Bladen sandy clay loam	234.58	3.9	3.71	372.96	a
Georgeville clay loam	428.1	3.2	7.45	522.48	b

[A]Parameters calculated using sorbed P (µg P/g) on the y-axis and solution concentration (µg/mL) on the x-axis.

[B]References were: (a) Ballard and Fiskell (1974), and (b) Fox and Kamprath (1970).

The fit of these models to the data is shown in Figure 2.5, and Figure 2.6. The Langmuir function generally increased too steeply at the lower concentrations of P in solution, then reached a plateau, apparently as the solid phase became saturated (Figure 2.5). The Freundlich function did not reach a plateau (Figure 2.6), and was more suited to the description of sorption curves than the Langmuir function.

Figure 2.5 - Langmuir fit of P sorption by soils of Ballard and Fiskell (1974), and Fox and Kamprath (1970).

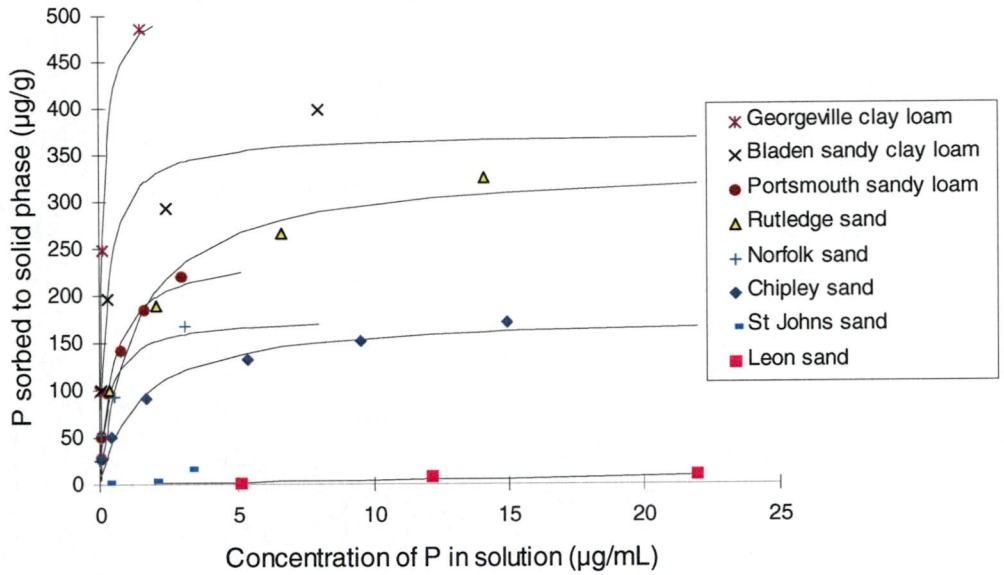
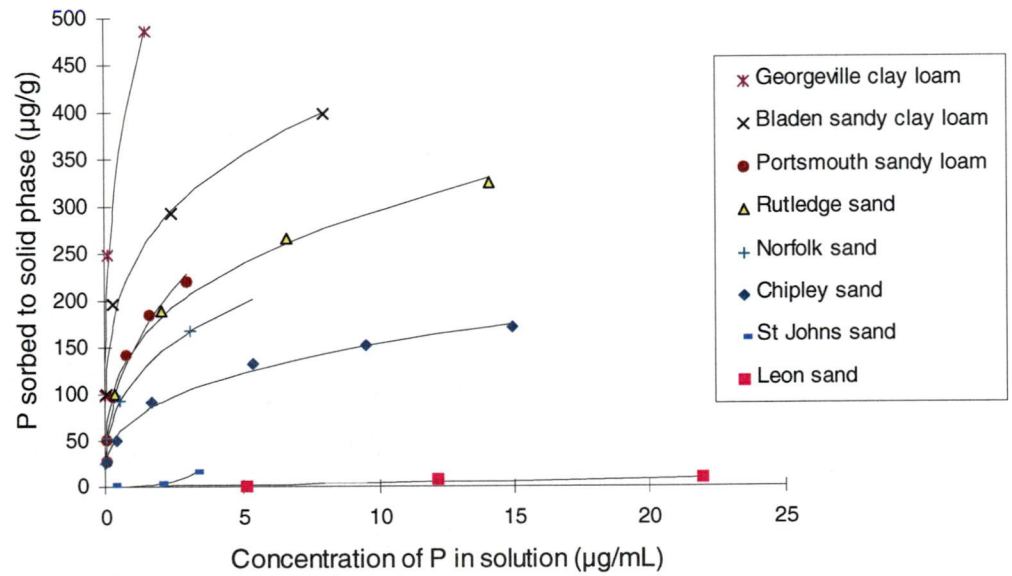


Figure 2.6 - Freundlich fit of P sorption by soils of Ballard and Fiskell (1974), and Fox and Kamprath (1970).



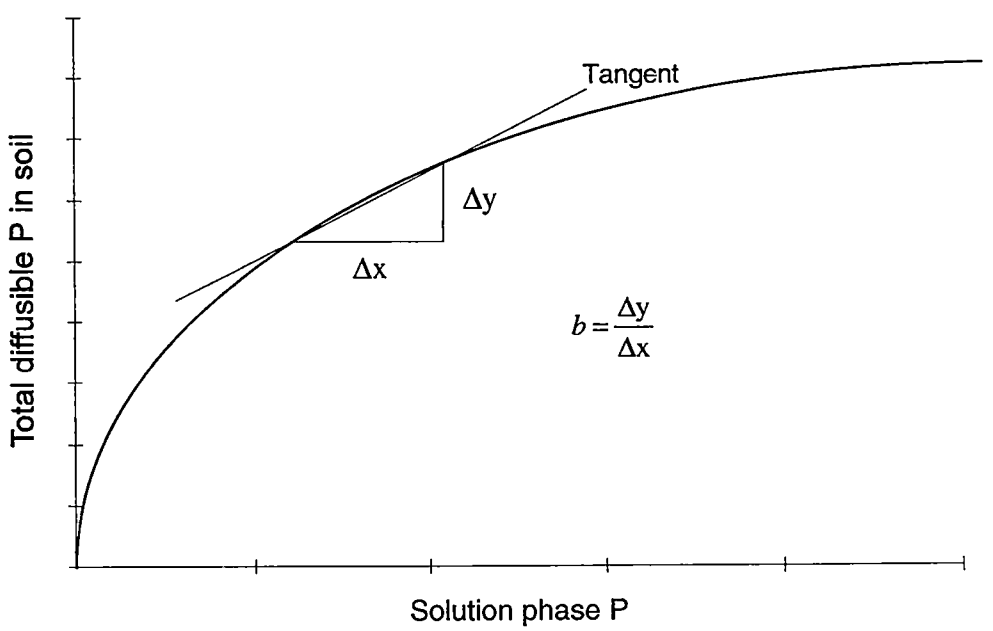
Barrow (1978) showed that the Freundlich function corresponded to a model of adsorption in

which the affinity term decreased exponentially as adsorption increased. Sposito (1980) showed that the Freundlich function corresponded to a model of surface adsorption to a number of heterogeneously mixed exchange surfaces, where sorption to each individual class of exchange surface was described by the Langmuir function.

2.2.2.1 Buffer power

Nye and Tinker (1977) defined buffer power (b) as the first derivative of the relationship between the concentration of total diffusible P in soil (C_t) and the concentration of P in the liquid phase (C_l), see Figure 2.7.

Figure 2.7 - Hypothetical P sorption curve.



Total diffusible P (C_t) was defined as the sum of P in solution (C_l) plus P on the solid phase (C_s) at a given solution concentration;

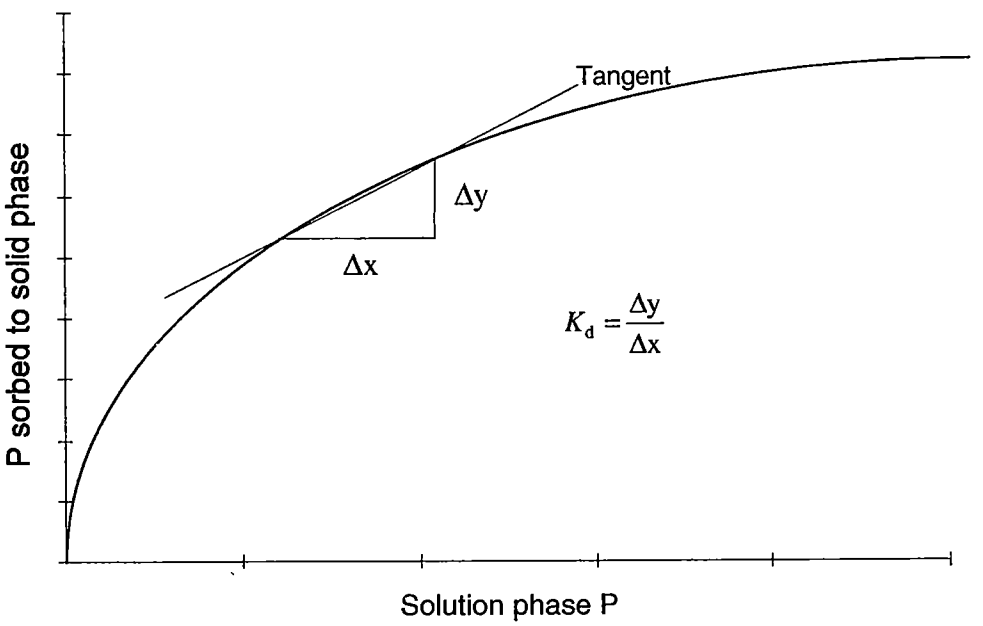
$$C_t = \theta_V C_l + \rho C_s, \qquad \text{Equation 2.1}$$

where θ_v is volumetric water content, and ρ is soil bulk density. Barber (1984), however, defined buffer power as the first derivative of the relationship between the concentration of

sorbed P on the solid phase (C_s), and the concentration in the liquid phase (C_l). This has also been referred to as the partitioning coefficient, or K_d (Equation 2.2, Figure 2.8).

$$K_d = \frac{dC_s}{dC_l}, \qquad \text{Equation 2.2}$$

Figure 2.8 - Hypothetical sorbed P vs solution P curve, showing calculation of the K_d value.



Van Rees *et al.* (1990b) showed that buffer power was defined by Equation 2.3,

$$b = \theta_v + \rho K_d, \qquad \text{Equation 2.3}$$

and also showed that soil water content was only important when the partition coefficient (K_d) was low. Hence, either definition of buffer power is likely to be useful for predicting P diffusion and nutrient replenishment to soil solutions, unless soil P is poorly buffered, such as with the Pomona sand of Smethurst and Comerford (1993a), or the Leon sand (Table 2.1) of Ballard and Fiskell (1974).

The *quantity* (or capacity) component of soil P is equivalent to the labile pool of soil P, and is the total amount of diffusible P in soil (C_t), while the *intensity* component is that in solution

(C_l).

2.2.2.2 Adsorption vs Desorption

The K_d of desorption curves is often lower than that of adsorption curves. This effect is called hysteresis, and has led researchers to the conclusion that adsorption reactions are irreversable (Olsen and Khasawneh 1980). However, Barrow (1983b) showed that while sorption is a reversible process, full reversibility is not observed in short-term sorption experiments, because of the slow nature of adsorption. A typical desorption experiment involves 'equilibrating' a soil with P, then using different soil:solution ratios to induce desorption. However, the initial addition of P does not equilibrate (due to the nature of slow sorption), and adsorption still occurs during the desorption experiment. Hence, the resultant desorption curve is actually the combination of further adsorption, as well as the desired desorption, and a lower K_d value results.

Hence, slow sorption influences plant nutrient availability in several ways: In soils with recently added P fertilizer, slow adsorption is likely to occur during growth of the plant, such that P desorption in the rhizosphere of plant roots is likely to have a lower K_d than that measured in a standard (17 hour) adsorption curve. However, in soils that have not been fertilized recently (ie. they are in a quasi-equilibrium), slow desorption would result in a higher K_d than that measured with standard adsorption curve methodology.

2.2.3 Relationships between P sorption and plant growth

Soil P buffer capacity, when combined with other indicators of P availability, improved the relationship between test results and yield response in ryegrass (Holford and Mattingly 1976) and wheat (Holford and Cullis 1985, Bolland *et al.* 1994). For example, buffer capacity accounted for 50% of the variance in the yield response curvature of wheat in 39 field experiments from north-western NSW (Holford and Cullis 1985). Yield response curvature was dependent on soil and crop type in these studies, which precludes widespread use in

other situations.

A number of studies have shown that plant growth is highly correlated with the concentration of P in solution (Section 2.3.2), and sorption curves could be used to predict the amount of fertilizer required to raise the concentration of P in solution to a level which produced optimal plant growth (Fox 1980).

Sorption curves have also been used extensively in plant nutrient uptake modeling to determine partitioning of P between solid and liquid phases (Nye and Tinker 1977, Barber 1984). However, sorption curves developed from short-term studies have limited applicability because they fail to account for longer term, or 'slow' sorption (eg. Barrow 1983a). Hence predictions of P fertilizer requirement and uptake over periods longer than that of the sorption curve are not estimated correctly. To overcome this problem a mathematical description of P sorption should incorporate a temporal component. Barrow (1987) showed that higher incubation temperatures could be used to accelerate the sorption process, and subsequently developed a model to account for long term sorption.

The intersection of a sorption curve with the x-axis provides an independent estimate of the concentration of P in soil solution, and is termed the equilibrium phosphorus concentration (EPC, Moody *et al.* 1988). At this concentration, P is neither sorbed nor desorbed from the solid phase.

2.2.4 Nutrient supply to the root surface

Nutrients are supplied to plant roots via interception, mass flow, and diffusion (Barber 1984). Root interception is the process whereby roots enter pores previously occupied by soil solution. Nutrients taken up by such a method do not move through the soil before being taken up by the plant root. Contributions of root interception to nutrient uptake are generally less than 5%. Mass flow is the movement of soil solution (and accompanying dissolved solutes), and is driven by transpiration. This process accounts for most of the supply of

nutrients that are highly mobile in the soil (eg. nitrate). Nutrient diffusion down a concentration gradient is the main mechanism of supply of nutrients that are poorly mobile in soil, such as P. The concentration gradient develops when uptake at the root surface exceeds supply of the nutrient by mass flow. The relative contribution of each of these mechanisms for nutrient uptake by maize in a fertile soil was calculated by Barber (1984), and reproduced here in Table 2.2. Mass flow provided most of the requirements for nutrients that were mobile in soil, such as nitrogen (79%, in that instance), but diffusion was the main mechanism of supply for relatively immobile nutrients, particularly P.

Table 2.2 - Relative contribution of root interception, mass flow and diffusion in supplying P, N and K to maize in a fertile soil, after Barber (1984).

	Percent contribution of:		
Nutrient	Root interception	Mass flow	Diffusion
Phosphorus	2.5	5	92.5
Nitrogen	1	79	20
Potassium	2	18	80

Nutrient diffusion to the root surface occurs as a depletion zone develops around the root. The depletion zone develops when the rate of uptake of ions exceeds the rate of supply by mass flow (Marschner 1995). After several days, a quasi-equilibrium is established, whereby nutrient uptake at the root surface maintains a lower concentration in solution at the root surface. The concentration gradient drives the diffusion mechanism. Effective diffusion in soil is described by Equation 2.4:

$$D_e = D_l \theta_v f \frac{1}{b}, \qquad \text{Equation 2.4}$$

Nye and Tinker (1977), where D_l is the diffusion coefficient of the nutrient in water, θ_v is the volumetric soil water content, f is the impedance factor (related to the tortuosity of the pathway that the ion takes in soil), and b is the buffer power of the soil (Section 2.2.2.1). Hence, effective diffusion of a nutrient to the root surface is directly related to soil water content, but inversely related to soil buffer power. Mechanistic models of these processes can be used to calculate the width of the depletion zone and the concentration at the root surface (eg. Nye and Tinker 1977, Barber 1984, Smethurst and Comerford 1993a).

2.3 Plant acquisition of phosphorus

Solution culture provides a homogenous root environment, which allows accurate control and/or measurement of root and solution parameters, such as pH, pO_2, E_h, nutrient ion concentrations and temperature (Asher and Edwards 1983), so it has been extensively used for investigating mineral nutrition of plants. Early research into phosphorus requirements of plants in solution culture found that initial concentrations of P in solution needed to be much greater than those required in soil for optimum growth of the plant (see Asher and Loneragan 1967). It was hypothesised that forms other than soil solution P were available to the plant (Burd 1947), but the cause of the requirement for high initial concentrations was actually the poor buffering ability of solution culture systems (Ingestad 1982). Asher and Loneragan (1967), and Edwards and Asher (1974) showed that the poor buffering ability of conventional solution culture experiments could be overcome by using large volumes of nutrient solution in flowing solution culture. They showed that soil solution was capable of directly providing the phosphorus requirements of plants. It is now accepted that inorganic P in soil solution is the main form of P taken up across the plasma membrane of root cells (Jungk 1991, Marschner 1995). Two main approaches have been taken for studying nutrient uptake in solution culture, these being the nutrient flux density (Ingestad 1971), and external concentration (eg. Asher and Loneragan 1967) approaches. Both approaches attempt to mimic the situation in soil, which generally has a large reservoir and a high buffering

capacity, compared with solution culture.

2.3.1 Nutrient flux density approach

Because of the shortcomings of conventional solution culture techniques the 'programmed nutrient addition' approach (Asher and Cowie 1970) and a refinement, the 'relative addition rate' approach (Ingestad 1971), were developed to study plant nutrient requirements. These systems used a small volume of nutrient solution with frequent additions of nutrients (Ingestad 1971). The nutrient under investigation was provided at sub-optimal levels, while other nutrients were provided at non-growth-limiting levels. Nutrient additions increased exponentially with time (Ingestad 1971), or at other programmed rates (Asher and Cowie 1970). Exponential rates of up to eg. 0.25 /day (ie. each day the plants accumulated 25% more dry matter) have been tested by Ingestad (1971). A reasonably constant internal concentration of the limiting nutrient was maintained in the plant with this system. The nutrient flux density approach has been utilized extensively to determine maximum seedling growth rates and optimum tissue concentrations and ratios of a range of nutrients in several plant species (eg. Ericsson and Ingestad 1988, Burgess 1991, Kirschbaum *et al.* 1992, Ericsson and Kähr 1993). However, such an approach is not useful for studies in which external concentrations need to be controlled (Asher and Edwards 1983), and is of minimal use for developing a soil test based on external concentrations of P. In a justification of the nutrient flux density approach, Ingestad (1982) calculated that flow rates of up to 80 L/min were required in flowing solution culture to maintain high growth rates (0.25 / day) of birch after 28 days of growth. In calculating that flow rate, Ingestad (1982) assumed 50% depletion of a 2 μM N solution (ie. 1 μmol of N per litre of solution available to the plant). The assumption that growth rates of 0.25/day could be achieved at an external concentration of 2 μM was not justified, because there are physical (diffusion) and physiological (uptake mechanism) limits to high uptake rates at low concentrations. The uptake ability of plant roots at 2 μM would limit growth rate to well below 0.25 /day.

Sands and Smethurst (1995) showed that the steady-state external concentration was about 1700 μM in the 0.25 /day treatments that Ingestad (1982) quoted above. Assuming that 5% depletion was acceptable (ie. about 85 μmoles/L), the flow rate required to maintain this concentration at the root surface would be about 1 L/min, supporting the statement of Edwards and Asher (1974), that 'flow rates of 1 litre per pot per minute or greater may be required to prevent excessive depletion of the nutrient solution'. Although flowing solution culture is currently the most appropriate method to investigate external concentration requirements, Sands and Smethurst (1995) suggested that the nutrient-flux density approach could also be adapted to calculate a critical external concentration. The approach of Sands and Smethurst (1995) required knowledge of nutrient uptake kinetics, which vary with pretreatment concentration and plant growth stage, so the critical concentrations calculated by this method would only be as accurate as the estimates of uptake kinetic parameters.

2.3.2 External concentration approach

Another school of thought suggests that there is a critical concentration of a given nutrient, above which no response to that nutrient occurs (Fox 1981). The critical concentration is dependent on species, and is likely to depend on plant demand (which is controlled by growth rates etc.). This theory takes into account that physical and physiological processes may limit P uptake by plants at low concentrations, no matter how highly the solution is buffered.

The concentration of P required in the external solution for optimal growth has been investigated in a number of experiments. External requirements for P in solution (Table 2.3), and soil (Table 2.4) have been published for some species. Asher and Loneragan (1967) examining P utilization of 8 different pasture species in solution culture, found that the concentration of P required in solution for maximum growth varied from 1 μM for silver grass, through to 25 μM for flatweed and barrel medic. Temple-Smith and Menary (1977a) found optimum concentrations of 0.4-0.5 μM for cabbage, and 2-4 μM for lettuce.

A range of P requirements between crops has also been found in soil-based investigations.

For example, Fox (1981) found that cassava required only 0.16 μM P in soil solution, while lettuce required about 10 μM P in soil solution. The critical concentrations for a number of species (Table 2.4) were within the range 0.2 - 10 μM.

Table 2.3 - Published concentrations of P in solution culture required for maximum growth of different species.

Species	Solution concentration (μM)	Reference
Brassica oleracea	0.4-0.5	Temple-Smith & Menary (1977a)
Vulpia myuros	1	Asher & Loneragan (1967)
Erodium botrys	5	Asher & Loneragan (1967)
Lactuca sativa	2-4	Temple-Smith & Menary (1977a)
Trifolium subterraneum	5	Asher & Loneragan (1967)
Bromus rigidus	5	Asher & Loneragan (1967)
Arctotheca calendula	5-25	Asher & Loneragan (1967)
Hypochoeris glabra	25	Asher & Loneragan (1967)
Medicago tribuloides	25	Asher & Loneragan (1967)

Dear *et al.* (1992) investigated the soil solution phosphate requirements of subterranean clover on a range of soil types. They found that 3.4 μM P was required in soil solution in a pot experiment with a range of soils, and 4.1 μM P was required in a range of field experiments. The minor difference in optimal concentration between the two types of experiments was remarkable, given the difference in culture conditions and duration. The

plants were grown for 7 weeks in the glasshouse experiment, and approximately 3 months for the field experiment. Plants in the field would have a higher critical concentration, because of a lower average soil water content (the soils in the glasshouse experiment were maintained at field capacity, while the water content in the field experiments would have been more variable). The critical concentration found by Dear *et al.* (1992) for subterraneum clover in the field (4.1 μM) was also the same as the critical concentration of P in soil solution for the predominantly subterranean clover 'pasture species' of Ozanne and Shaw (1967). Hence, the concentration of phosphorus in soil solution was well correlated with growth of subterranean clover across a range of soil types. The concentration of P required in soil solution for optimal growth of two related *Pinus* species (*Pinus radiata* and *Pinus taeda*) may also be similar, as both showed a very similar requirement for P in soil solution in two different experiments (Table 2.4, Tiarks 1982, Skinner and Attiwill 1981).

The P requirements for optimum growth of lettuces were different in solution (3 μM, Temple-Smith and Menary 1977a), and soil (10 μM, Fox 1981). Some of the difference may have been genetic, for example, Nagata *et al.* (1992) found a significant cultivar x P rate interaction for marketable yield of lettuce varieties. Another likely reason was that the concentration at the root surface in soil would have been less than that in bulk soil solution, due to the P depletion gradient that develops around roots growing in soil (Nye and Tinker 1977). The concentration of P in solution at the root surface may have approached 3 μM P in the experiment cited by Fox (1981). The concentration of P required at the root surface in soil may be equivalent to the critical concentration required in solution culture for growth. A soil independent test for P may possibly be developed by using the models described in Section 2.3.7 to predict the concentration of P at the root surface.

Table 2.4 - Critical soil solution concentration of P found in soil-based experiments.

Species		Critical growth level[^]	Solution concentration (μM)	Reference
Trifolium subterraneum	Pot experiment	90	3.32	Dear et al. (1992)
Pinus taeda	Pot experiment	90	6.14	Tiarks (1982)
Pinus radiata	Pot experiment	~100	6.5	Skinner & Attiwill (1981)
Manihot esculenta	Field experiment	95	0.16	Fox (1981)
Panicum maximum	Field experiment	90	0.9	Moody & Standley (1980)
Melinis minutiflora	Field experiment	90	1.1	Moody & Standley (1980)
Stylosanthes guianensis	Field experiment	90	1.2	Moody & Standley (1980)
Brassica oleracia	Field experiment	95	1.29	Fox (1981)
Centrosema pubescens	Field experiment	90	1.6	Moody & Standley (1980)
Zea mays	Field experiment	95	1.62	Fox (1981)
Sorghum bicolor	Field experiment	95	1.94	Fox (1981)
Pasture spp.	Field experiment	90	4.12	Ozanne & Shaw (1967)
Trifolium subterraneum	Field experiment	90	4.13	Dear et al. (1992)
Glycine max	Field experiment	95	6.46	Fox (1981)
Lycopersicon esculentum	Field experiment	95	6.46	Fox (1981)
Lactuca sativa	Field experiment	95	9.69	Fox (1981)

[^] The critical level for growth is represented as a percentage of the maximum growth; the different levels used by different authors are indicated in this column.

2.3.3 Root morphology

Ion and water transport through the cortex of the root can be apoplastic (through the cell walls and intercellular spaces), and/or symplastic (through the cell cytoplasm). Apoplastic movement of water and anions occurs in the water free space (WFS), which is a component of the apparent free space (AFS) of Hope and Stevens (1952). The effects of the AFS on uptake are discussed further in Section 2.3.5. Apoplastic movement of water and ions is

prevented at the endodermis by the casparian band (CB), which is a band of hydrophobic compounds (suberin and lignin) around the stele. Free water entry can occur (a) at the root tip (prior to formation of the CB), (b) where the CB is transiently ruptured by the formation of lateral roots (Marschner 1995), and (c) through passage cells, which are a small proportion of unsuberised cells crossing the CB in some species (Clarkson 1991). However, the casparian band is intact along the majority of the length of a root system, so symplastic transport is the main mechanism for ion movement into the stele. For symplastic transport to occur, nutrient ions enter root epidermal or cortical cells by crossing the plasma membrane. Uptake of P_i across the plasma membrane is an active process against a large concentration gradient. Phosphorus in the cell cytoplasm can be 10^3 to 10^4 times more concentrated than that in solution outside the cell (Bieleski 1973, Clarkson and Grignon 1991). The mechanism of uptake across the plasma membrane is described further in Section 2.3.4. Once P has entered a cortical or epidermal cell, symplastic transport toward the stele occurs via plasmodesmata, which are cytoplasmic bridges between cells (Marschner 1995). The absorbing surface of young roots in solution culture is equivalent to the surface area of all of the cells in the epidermis and cortex. In soil, uptake may be restricted to the outer cells, especially for ions that are poorly mobile (such as phosphate), because diffusion of ions from dilute solutions into the apoplast may be slower than absorption by the outer cells (Clarkson 1991). This hypothesis was supported by Hay *et al.* (1986), who investigated P uptake of unthickened and secondarily thickened *Trifolium repens* roots. Phosphorus uptake on a per-unit length basis was similar for both root types, even though epidermal and cortical cells were not present in the secondarily thickened roots (the epidermis was replaced by a periderm). Translocation from secondarily thickened roots was lower, probably because of suberization of multiple layers of periderm in the thickened roots. Suberized roots of *Pinus taeda* were also shown by Chung and Kramer (1975) to contribute significantly to P uptake, although the rate of uptake by suberized roots was 32% of the rate by unsuberized roots. Again, the difference was probably due to slower transport through the suberized layers of cells.

By electrostatic repulsion, fixed negative charges present in cell walls would also reduce penetration of the negatively charged phosphate ion into the AFS. For example, Rufty *et al.* (1986) bathed maize roots in nitrate solutions (0.2 mM and 20 mM) for 20 hours, and then used a microsurgical technique to separate cells of the epidermis, cortex and stele. Uptake of NO_3^- from both solution concentrations was confined mainly to the epidermal cells (as measured by a nitrate reductase assay), but there was some activity in the other cell types at the higher pre-treatment concentration.

Barber and Silberbush (1984) investigated the effect of root length and diameter in a sensitivity analysis of a mechanistic uptake model. Predicted P uptake increased linearly with root length, and also with root radius. Barber and Silberbush (1984) also used the mechanistic model to investigate the most effective allocation of carbon to roots for the purposes of P uptake. They hypothesised that for a given amount of carbon allocated to the root system, the plant could either produce a short length of thick roots, or a longer length of thin roots. Roots with smaller radii, but longer length had a higher predicted uptake, but the advantages of longer root length reduced as depletion zones around the roots started overlapping at high root density. Barber and Silberbush (1984) did not account for variable uptake parameters with different root radii in their model, but the surface area for absorption (ie. the number of transporters) could vary markedly with root radius. The surface area for absorption would depend on the penetration of phosphate into the root cortex. If the concentration of P in the AFS did not change radially, the uptake ability of the roots would increase with the square of the root radius (volume for absorption = $\pi r^2 l$). The optimal root radius would depend on the gradient in concentration of P within the AFS, about which little is known.

Root hairs were thought to be an important attribute for uptake, because they increased the effective radius of the root at low carbon cost (Nye and Tinker 1977). This hypothesis was not supported by Bole (1973), who found no close relationship between P uptake and root hair density, or by Temple-Smith and Menary (1977b), who used P-32 autoradiography to examine the depletion zones of P around lettuce and cabbage roots. Temple-Smith and

Menary (1977b) found that the diameter of the depletion zone was less than the diameter of the root hair cylinder in a highly P-sorbing soil. This observation supported an alternative theory, that root hairs increase the surface area for absorption. Clarkson (1991) calculated that root hairs could increase the surface area of the root 3-fold, with only a 2% increase in dry weight. It was thought that root hairs were especially important for elements like P that are poorly mobile in the soil, because they can extend beyond the depletion zone around the root. Föhse *et al.* (1988, 1991) developed a model to explain the location of P uptake into several plant species. They found that root hairs accounted for up to 90% of the influx, because the concentration of P at their uptake surface decreased at a slower rate than around the main root (due their small radius), and therefore the P influx remained higher. Such an hypothesis has not been observed using more direct methods such as P-32 autoradiography, possibly because the resolution of such a method may not be high enough to observe the relatively small depletion zones that are hypothesised to occur around root hairs. Gahoonia *et al.* (1997) showed that varieties of wheat and barley with more root hairs depleted a greater amount of P from soil than those with fewer root hairs, especially at lower levels of soil P. Caradus (1981) showed that long-haired white clover populations had greater growth and P uptake than populations with shorter root hairs, but the benefit of root hairs was minimal at either high or low levels of soil P. The efficiency of root hairs in P acquisition is also dependent on species, for example, only broad trends between root hairs and P uptake efficiency of seven species were found by Föhse *et al.* (1988, 1991). Spinach had the highest root hair density, longest root hairs and correspondingly, the highest influx of P. Ryegrass, wheat and rape all had similar root hair lengths, but P influx differed four-fold between them. The main contribution of root hairs was not to increase the size of the depletion zone around the main body of the root, but to extend beyond the depletion zone, and take up P from the zone of highest concentration in solution.

Root hairs of *Trianea bototensis* and *Raphanus sativus* have a greater number of plasmodesmatal connections than neighbouring epidermal cells (Vakhmistrov 1981),

supporting the hypothesis that root hairs are involved in nutrient transport.

Hence, root hairs play an important role in P uptake, but the actual mechanism may be dependent on interactions between plant species and soil characteristics.

2.3.4 Kinetics of P uptake

Epstein and Hagen (1952) and Epstein (1953) elucidated the carrier concept of nutrient uptake, whereby they hypothesised that uptake across the plasma membrane was mediated by enzyme carriers. They used the theory of simple enzymic catalysis (Michaelis-Menten kinetics) to describe the rate of reaction, where the rate of activity (I) of the carrier complex was given by Equation 2.5:

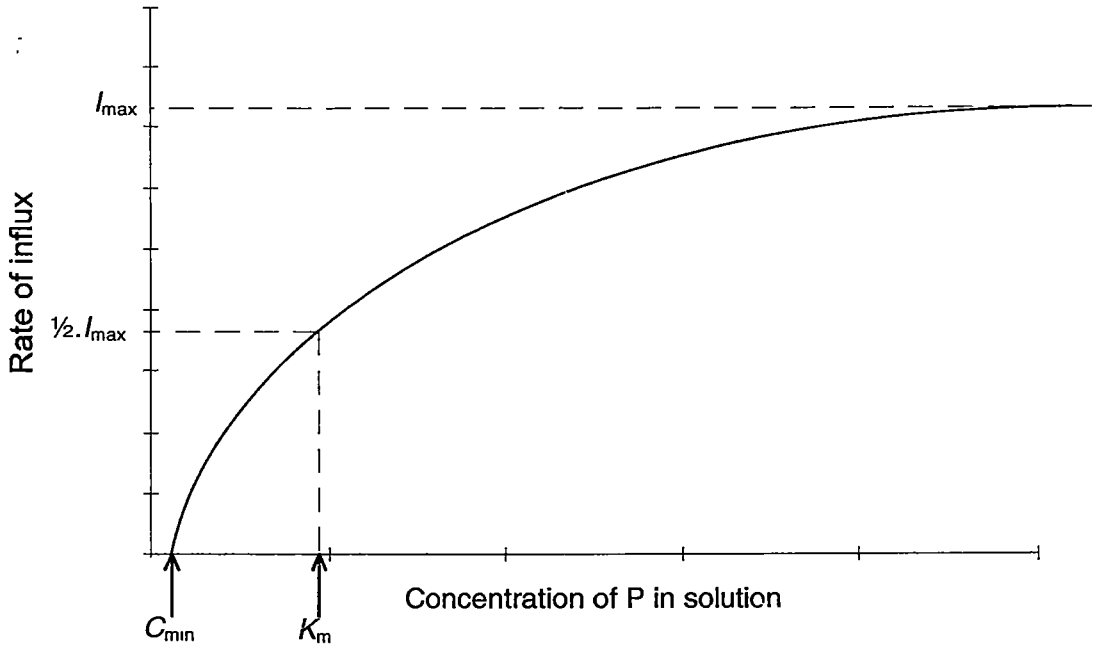
$$I = \frac{I_{max} \cdot C_l}{K_m + C_l}, \qquad \text{Equation 2.5}$$

where C_l was the concentration of the nutrient in the bathing solution, I_{max} was the rate of maximal influx (which occurred when the protein complex became saturated), and K_m (or Michaelis constant), was equal to the concentration in solution at half I_{max}. Neilsen and Barber (1978) modified the equation to include a C_{min} parameter (Equation 2.6), where C_{min} was the concentration at which net influx was zero.

$$I = \frac{I_{max} \cdot (C_s - C_{min})}{K_m + (C_s - C_{min})} \qquad \text{Equation 2.6}$$

A typical influx curve of the high affinity transport system is shown in Figure 2.9, with influx on the y axis (eg. nmol/g fresh weight/hour), and concentration in the external solution on the x axis (eg. μM).

Figure 2.9 - Derivation of the kinetic parameters on a hypothetical nutrient influx curve.



The value of I_{max} is thought to be influenced by the number of carriers (transporters) per unit length of root (Clarkson 1985). The K_m parameter is thought to be an indicator of the affinity of the uptake mechanism, i.e. the lower the K_m, the higher the affinity that the carrier mechanism has for that ion.

The molecular basis of ion transport over the full concentration range of uptake is unknown, but the first phase of ion transport (at lower concentrations in the external solution) is mediated by a carrier-type mechanism. At low concentrations of P (typically those in soil solution), the relationship between uptake and external concentration can be described by Michaelis-Menten kinetics. At higher concentrations, uptake follows a near-linear or multiphasic type of mechanism.

One of the simplest models of the molecular basis of uptake was a carrier + diffusion model, whereby uptake at low concentrations was considered to be an active process, but at higher

concentrations, the 'linear' component of uptake was due to passive influx of the nutrient. This model was proposed for K^+ uptake by maize roots (Kochian and Lucas 1982). The carrier + diffusion model would not be the mechanism of phosphate uptake, due to the large difference in P concentration between that in the external solution and that in the cell. For example, Lefebvre and Clarkson (1984b) found that the first phase of uptake in pea root protoplasts had a maximal influx of 1990 μmoles/g FW/hour, and a K_m of 9.9 μM. That mechanism would be saturated at approximately 90 μM in the external solution, but the concentration in the cytoplasm of the cell is likely to be 10-100 fold greater than that concentration (Bieleski 1973, Clarkson and Grignon 1991). Hence for phosphate, the diffusion model is unlikely to be the explanation for observed 'linear' uptake.

Other models of P transport across the plasma-membrane incorporated a dual uptake mechanism (Epstein 1976) with a high affinity transport system (HATS), which corresponds to the first phase of uptake at low concentrations, and a separate molecular entity controlling the low affinity transport system (LATS). The LATS was hypothesised to be due to either (a) active transport via a carrier, (b) transport down an electrochemical gradient via ion channels, (c) passive diffusion, or a combination of all three mechanisms. Nutrients with a relatively high concentration in soil solution, for example potassium (typically 25 - 2000 μM, Tisdale *et al.* 1993), probably flow through ion channels. Maathuis and Sanders (1996), using patch-clamped root cells of *Arabidopsis thaliana*, showed that ion channels were important for K^+ uptake by the LATS, but again this mechanism is unlikely for phosphate, due to the large concentration difference across the plasma membrane.

The concept of multiphasic uptake was developed by Nissen and co-workers (eg. Nissen 1971, Nissen *et al.* 1980, Nissen 1980, 1989,1991, Nissen and Nissen 1983). Nissen (1991) hypothesised that uptake was due to a single mechanism or transport structure residing in the plasma membrane, with different phases of uptake caused by different conformations of the one structure. A single structure model was preferred, because of the large number of phases, and apparent integration between them (ie. the end of one phase coincided with the beginning

of the next phase). The transport mechanism was hypothesised to act like a carrier (ie. low rate and high specificity) at low external concentrations, and as an ion channel (ie. high rate, low specificity) at higher external concentrations. The multiphasic model has been interpreted for a number of different data-sets (Nissen 1991), but the phases in many of those experiments appear to begin and end in aberrations of a linear trend, or they are described by very few points. The theory of Nissen (1991) is compatible with observed P uptake characteristics, but the experimental evidence is equivocal in many cases.

The first phase of uptake (corresponding to the HATS) is the most important for phosphate uptake from soil, because it covers the range of concentrations that commonly exist at the root surface in soil.

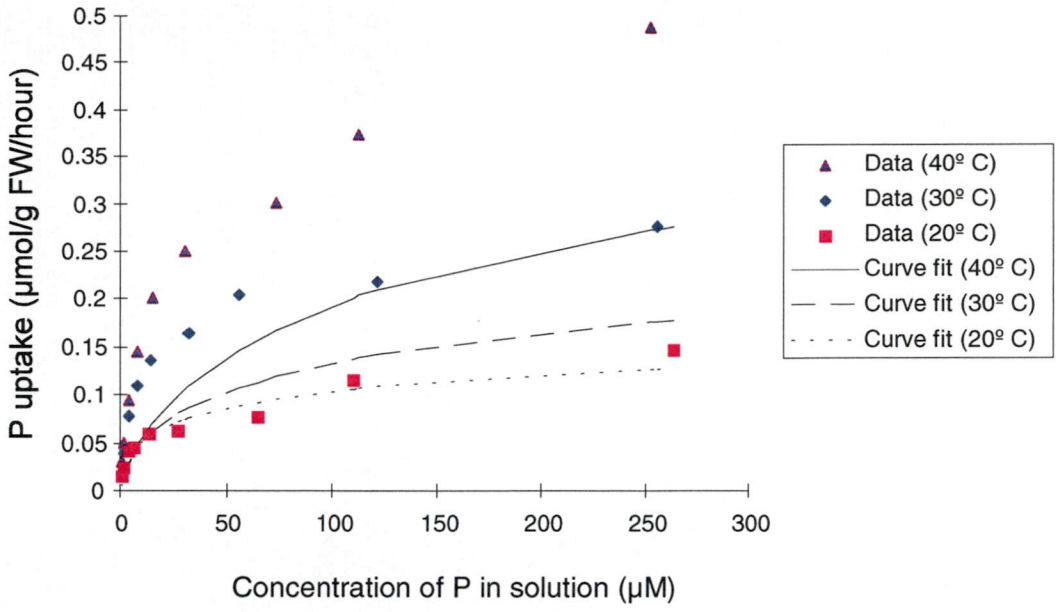
Radioisotopes such as P-32 have been extensively used to characterize the short-term ability of plant roots to take up phosphorus. Such studies are particularly useful for this purpose, as they allow measurements on excised roots, which do not have the complications of root and shoot feedback mechanisms (Huang *et al.* 1992). Conversely, evidence also exists that root processes are severely disturbed by excision. For example, net fluxes of nutrients such as NH_4^+, K^+ and NO_3^- are reduced within 2 hours of excision (Bloom 1989). Garnett (1996) found that NH_4^+, NO_3^-, and H^+ fluxes in *E. nitens* roots reduced by approximately 80%, 45 minutes after excision. These studies were conducted on whole-plants, and measured fluxes were a result of both influx and efflux. Plants in both studies were pretreated at the same concentrations in which the flux measurements were taken, so excision of the shoot probably reduced demand, and consequently uptake. Net P influx (ie. without efflux) is measured by the radiotracer method of Epstein *et al.* 1963, because the cellular (ie. cytoplasmic/vacuolar) pools of P are reduced by a 12-24 hour pretreatment in a P-free solution. Hence, the carrier mechanism continues functioning after excision because the cytoplasmic and vacuolar pools are low in P, and create a demand for it. Additionally, the relatively short duration of radiotracer experiments (less than 1 hour) is within the time period during which the effects of excision were minimal in the study of Bloom (1989), and P uptake is little affected by root

excision (Gronewald and Hanson 1982), compared with other nutrients such as NO_3^- and NH_4^+. Methodology for radioisotope uptake by excised roots was introduced by Epstein (1953) and recently validated by Huang *et al.* (1992).

Published kinetic parameters of the high affinity transport mechanism of P uptake vary widely (Table 2.5). Some of the differences reported between species are probably genetic, but the values for kinetic parameters also vary with experimental methods.

Methods of linearization of Michaelis-Menten uptake data were developed to allow calculation of the non-linear parameters (Eadie 1942, Hofstee 1952). Application of these methodologies was problematic, and kinetic parameters obtained using these methods were sometimes poor fits of the original concentration dependent uptake data. For example, Carter and Lathwell (1967) used a Hofstee plot to identify the 'a' and 'b' components of uptake (ie. low and high affinity mechanisms, respectively), but the kinetic parameters that they obtained fitted the data poorly for the 30°C and 40°C treatments (Figure 2.10). The values in Figure 2.10 were measured from Figure 2 of Carter and Lathwell (1967), and the fitted curves were calculated by summing uptake predicted by the 'a' and 'b' mechanisms given by Carter and Lathwell (1967).

Figure 2.10 - Data and derived curves of Carter and Lathwell (1967)

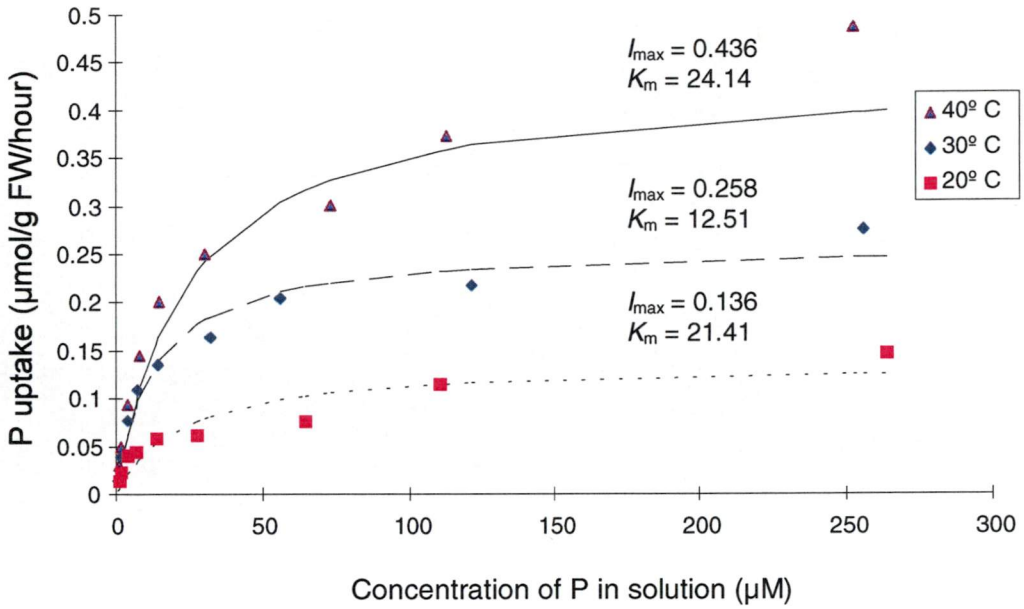


More appropriate models can be fitted directly by using curve fitting software. A single phase Michaelis-Menten model fitted the data of Carter and Lathwell (1967) better than those originally described (Figure 2.11). There are many examples where methods of curve fitting have led to erroneous estimation of I_{max} and K_m. Garnett (1996) reanalysed kinetic data from a number of published studies, and concluded that methods of linearization often gave poor estimates of kinetic parameters.

The new I_{max} parameters from the reanalysis of the Carter and Lathwell (1967) data (ie. 0.136, 0.258 and 0.436 µmoles/g FW/hour for the 20°C, 30°C and 40°C treatments, respectively) were similar to the I_{max} of the high affinity mechanism for P uptake in *Zea mays* reported by Jungk *et al.* (1990; Table 2.5). Jungk *et al.* (1990) measured uptake kinetics using depletion-type experiments on whole plants, whereas Carter and Lathwell (1967) measured the parameters in short-term (1 hour) excised-root studies, so the degree of similarity between the values for the I_{max} coefficient was remarkable. Contrary to the conclusions of Carter and Lathwell, there was no apparent unidirectional trend in K_m with temperature, although the roots seemed to have the highest affinity (ie. lowest K_m) at 30°C, which may be indicative of

the optimum temperature for P uptake by maize roots.

Figure 2.11 - Re-analysed Michaelis-Menten curves for the data of Carter and Lathwell (1967)



Pretreatment concentration had a minor and inconsistent effect on the K_m of maize and soybeans (Jungk *et al.* 1990), wheat (Cogliatti and Santa Maria 1990) and *Arabidopsis* (Dunlop *et al.* 1997), but increasing P pretreatment concentration significantly and consistently decreased I_{max}. Increased I_{max} in conditions of low P availability is a mechanism used by plants to increase their ability to acquire P. Clarkson and Grignon (1991) hypothesised that *de novo* synthesis of transport proteins caused changes in uptake ability of plant roots over time-scales of a few days, and that theory was consistent with the observed effect of different pretreatment concentrations on the value of I_{max}.

Table 2.5 - Published values of kinetic parameters for the high affinity P uptake mechanism of several species and different pretreatment conditions.

Plant	I_{max} (μmol/g FW/hour)	K_m (μM)	C_{min} (μM)	Note	Reference
Pinus radiata	0.436[A]	2.9	-	0 ppm NO_3^- pretreat.	Taber and McFee (1972)
Pinus radiata	0.545[A]	3.3	-	100 ppm NO_3^- pretreat.	Taber and McFee (1972)
Triticum aestivum	1.15[B]	26	-	50 μM pretreat.	Cogliatti & Santa Maria (1990)
Triticum aestivum	0.96[B]	36.3	-	500 μM pretreat.	Cogliatti & Santa Maria (1990)
Triticum aestivum	0.55[B]	26.5	-	5000 μM pretreat.	Cogliatti & Santa Maria (1990)
Glycine max	0.455[A]	1.6	0.01	0.03 μM pretreat.	Jungk *et al.* (1990)
Glycine max	0.441[A]	1.7	0.03	0.3 μM pretreat.	Jungk *et al.* (1990)
Glycine max	0.166[A]	1.2	0.08	3 μM pretreat.	Jungk *et al.* (1990)
Glycine max	0.094[A]	1.0	0.06	30 μM pretreat.	Jungk *et al.* (1990)
Zea mays	0.962[A]	6.1	0.01	0.1 μM pretreat.	Jungk *et al.* (1990)
Zea mays	0.557[A]	3.9	0.02	1 μM pretreat.	Jungk *et al.* (1990)
Zea mays	0.174[A]	1.9	0.04	10 μM pretreat.	Jungk *et al.* (1990)
Zea mays	0.188[A]	3.4	0.02	100 μM pretreat.	Jungk *et al.* (1990)
Pinus taeda	?	16	0.6		Kelly *et al.* (1992)
Pisum sativum	?	9.9	-	protoplasts only	Lefebvre & Clarkson (1984b)
Lolium perenne	0.337	2.3	-		Temple-Smith (1973)
Phalaris tuberosa	0.266	3.3	-		Temple-Smith (1973)
Brassica oleracea	0.061	1.0	-	non-sterile	Temple-Smith & Menary (1974)
Brassica oleracea	0.031	1.1	-	sterile	Temple-Smith & Menary (1974)
Lactuca sativa	0.077	1.5	-	non-sterile	Temple-Smith & Menary (1974)
Lactuca sativa	0.065	1.7	-	sterile	Temple-Smith & Menary (1974)

[A] Calculated assuming a root diameter of 0.4 mm, and a root specific gravity of 1.1 g/cm^3

[B] Calculated assuming a root fresh weight:dry weight ratio of 10.

Temple-Smith and Menary (1974) investigated the effect of microbial inoculation on P uptake of cabbage and lettuce plants. They found that microbial inoculation approximately doubled the I_{max} of cabbage roots, but did not significantly affect the K_m. Microbial

inoculation did not affect either kinetic parameter of lettuce roots.

2.3.4.1 Reconciliation of the nutrient flux density and external concentration approaches

Sands and Smethurst (1995) showed that Michaelis-Menten kinetic theory was not inconsistent with uptake observed in the nutrient flux density experiments of Ingestad and Lund (1979). Sands and Smethurst (1995) used Michaelis-Menten kinetic theory to calculate concentration of N in each relative addition rate treatment of Ingestad and Lund (1979). The assumptions that Sands and Smethurst (1995) used were that (a) growth was nitrogen limited, (b) the plants were in a steady state (ie. that plant nitrogen concentration and partitioning to the roots was constant over the experimental period), and (c) nutrient uptake was based on Michaelis-Menten-like uptake kinetics. The model required assumption of the K_m parameter, and calculated the I_{max} parameter from the concentration at which relative growth rate became less than relative addition rate. The concentrations calculated by Sands and Smethurst (1995) were similar to those published by Ingestad and Lund (1979), indicating that Michaelis-Menten kinetics were not inconsistent with observed uptake in that system. However, the assumption that uptake was based solely on Michaelis-Menten kinetics may reduce applicability of this method for determining critical concentrations in solution in nutrient flux density experiments, because it only takes into account the high affinity mechanism. If the low affinity mechanism also contributed to uptake, assumption (c) may lead to an over-estimation of the I_{max} parameter. Another limitation was the requirement for assumption of K_m. Despite these limitations, such an approach could potentially be used to estimate P uptake characteristics of *Eucalyptus grandis* from a published P relative addition rate experiment (Kirschbaum *et al.* 1992).

2.3.4.2 Application of uptake kinetic parameters

Uptake kinetic parameters allow calculation of concentration-dependent uptake of nutrients by plant roots in nutrient uptake models (eg. Nye and Tinker 1977 and Barber 1984). Values of I_{max} and K_m vary with plant age and pretreatment concentration (Clarkson 1985), but the

effect that these variations have on predicted uptake may be reduced in some situations, because the values of I_{max} and K_m only had a minor influence on the calculated P uptake in the soil system (Silberbush and Barber 1983). The value of I_{max} changes with respect to the concentration of the nutrient in the pre-treatment (Jungk *et al.* 1990, Lee 1982), and this is thought to be due to changes in the number of carriers in the plasma membrane (Clarkson 1985). The change in K_m with P pretreatment concentration is not consistent, and K_m is thought to be a more intrinsic property of the carrier proteins, rather than a function of the number present. The C_{min} parameter is important for uptake modelling, because net nutrient uptake ceases below this concentration. At low concentrations in soil solution, the value of C_{min} has a large influence on predicted uptake.

It has also been suggested that plant growth will not be limited by nitrogen if the concentration of N at the root is around the K_m value (Clarkson 1985, Smart and Bloom 1993).

2.3.5 Changes in chemistry close to the root surface

Rhizosphere acidification is a strategy employed by many plants to increase the availability of P at the root surface. Geelhoed *et al.* (1997) showed that P release by goethite (FeOOH) increased with lower pH in suspension. Plant dry weight observed by Geelhoed *et al.* (1997) was 1.29, 1.68 and 3.08 at pH 5.5, 4.6 and 3.7, respectively, due to P release at lower pH's. Acidification causes an increase in competitive ability of other anions (eg. SO_4^{2-} and organic anions) for sorption sites, and consequently P is released from those sorption sites (Geelhoed *et al.* 1997, Marsh *et al.* 1992).

Grinsted *et al.* (1982) showed that the pH in the rhizosphere of *Brassica napus* decreased from 6.5 to 4.1 over a 35 day growing period. Greater cation than anion uptake is often the cause of rhizosphere acidification because charge balance is maintained within the root via proton efflux. The pH decrease observed by Grinsted *et al.* (1982) led to a commensurate 10-fold increase in the concentration of P in soil solution. Even though nitrate was the sole

source of nitrogen in that experiment, the cause of the pH decrease was an imbalance in the ratio of cations to anions taken up (Hedley *et al.* 1982b), rather than release of organic acids from either the roots or microorganisms in the rhizosphere. Rhizosphere acidification due to proton efflux (in response to greater cation than anion uptake) has been widely reported (Haynes 1990). Saleque and Kirk (1995) calculated that at least 80% of P taken up by lowland rice was made available to the plant via rhizosphere acidification (from a pH of 5.8 at 8 mm from the root surface, to ~4.2 at 1 mm from the root surface) and subsequent P release. Preferential uptake of ammonium (with associated extrusion of an H^+ ion) often leads to rhizosphere acidification (Nye 1981), and *E. nitens* is highly dependent on ammonium as the source of nitrogen (Garnett 1996). It could be speculated that part of the reason for the dependence of *E. nitens* on ammonium may be for P acquisition. Australian soils generally have very poor phosphorus availability (Beckman 1983), so acidification of the rhizosphere (via preferential uptake of ammonium) could potentially increase P availability at the root surface.

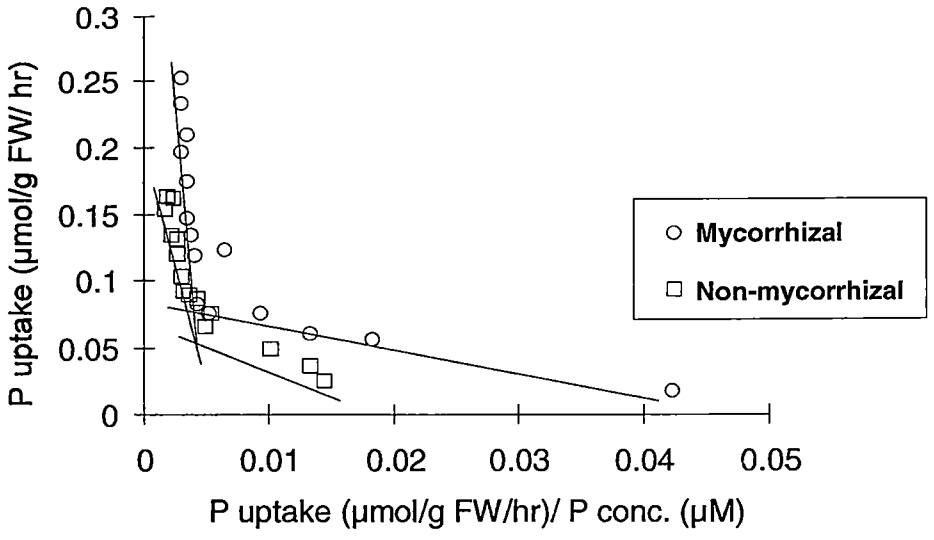
The microenvironment close to the plasma membrane also has a large influence on the ability of the plant roots to acquire P, mainly via control of charge and pH in the cell walls. Hope and Stevens (1952) showed that the apparent free space (AFS) in cell walls is comprised of water free space (WFS) and Donnan free space (DFS). The DFS is attributed to the permanent negative charges located in the cell walls, which gives the AFS a cation exchange capacity (CEC). The CEC attracts cations, and repels anions such as phosphate, which is thought to be the reason for the selectivity of roots for the $H_2PO_4^-$ ion over the dinegative HPO_4^{2-} ion (Clarkson and Grignon 1991). The relative charge on the cation exchange surfaces is reduced as the concentration of H^+ ions is increased (ie. with decreased pH), so the electrostatic repelling force is also reduced. Hence reduced pH in cell walls generally leads to increased P transport and uptake. The pH within cell walls close to the plasma membrane is partially buffered at an optimal level for P uptake by proton efflux from root cells.

2.3.6 Mechanism of mycorrhizal action

Various mechanisms of mycorrhizal action have been proposed to explain the increase in P uptake by plants infected with mycorrhizal fungi in soils of low P availability. The possible mechanisms were suggested by Bolan (1991) to be (a) exploration of a larger soil volume, due to small diameter mycorrhizal hyphae entering volumes of soil material unavailable to roots, or hyphae extending beyond the zone of depletion. The small diameter of mycorrhizal hyphae allows them to enter small pores in the soil, which have a higher water content than large pores, and hence greater P availability (Equation 2.4). The high length to diameter ratio of hyphae is an efficient use of assimilate to effectively increase the length of the root system, (b) increased uptake ability of mycorrhizae, or affinity of mycorrhizae for P, or (c) due to solubilization of otherwise unavailable forms of P via exudation of enzymes, such as phosphatases, which liberate P bound in organic matter.

One widely cited paper (Cress *et al.* 1979) investigated the first two of these hypotheses by examining the P uptake characteristics of mycorrhizal and non-mycorrhizal tomato (*Lycopersicon esculentum*) roots. They determined that mycorrhizal inoculation did not influence I_{max} of the high affinity uptake mechanism, but induced a lower the K_m, indicating a higher affinity for P with mycorrhizal inoculation (Table 2.6). Based on these observations, Cress *et al.* (1979) postulated that mycorrhizal infection increased the affinity of the root system for P at low concentrations, rather than increasing the surface area for absorption. The kinetic parameters in that paper were determined from Augustinsson-Hofstee plots, similar to those shown in Figure 2.12. These data were re-analysed by directly fitting a Michaelis-Menten model to an uptake vs concentration graph (Figure 2.13). Values for the reanalysis were derived by measuring the points on the graph presented by Cress *et al.* (1979).

Figure 2.12 - Augustinsson-Hofstee plot derived from Cress *et al.* (1979).



From comparison of the kinetic parameters obtained via these two methods (Table 2.6), it is clear that the conclusions arrived at by Cress *et al.* (1979) were not justified. Both the I_{max} and K_m coefficients increased with mycorrhizal inoculation, indicating that the mycorrhizal effect was probably due to increased surface area, rather than an enhanced affinity for phosphorus. This conclusion was supported by Li *et al.* (1991), who found that mycorrhizal hyphae alone depleted water extractable P to a similar or lesser extent than both roots and hyphae. Thomson *et al.* (1990) found that the high affinity transport system of a mycorrhizal fungus (*Gigaspora margarita)* had a K_m of 1.8-3.1 µM, which was within the range of K_m values for plants presented in Table 2.5.

Figure 2.13. Michaelis-Menten uptake curve for the high affinity concentration range up to 50 µM, derived from Cress *et al.* (1979).

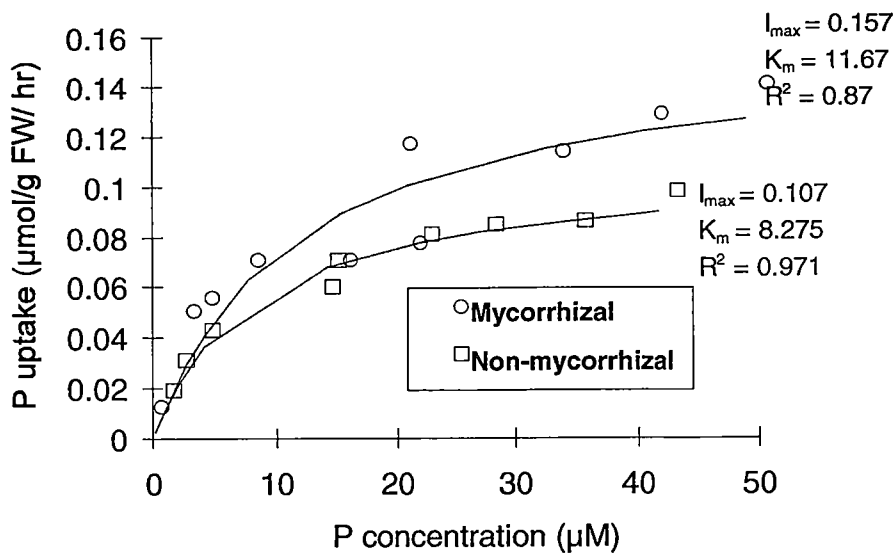


Table 2.6. Comparison of the high affinity I_{max} and K_m values obtained by Cress *et al.* (1979), and from a reanalysis of their data (Figure 2.13).

	I_{max} (µmol/g FW/hour)		K_m (µM)	
	Cress *et al.* (1979)	This analysis	Cress *et al.* (1979)	This analysis
Non-mycorrhizal	0.1*	0.107	3.9*	8.275
Mycorrhizal	0.1*	0.157	1.6*	11.67

* Note that the Cress *et al.* (1979) used the range 0-20 µM to obtain the high affinity kinetic parameters, but the 0-50 µM range is more appropriate (Figure 2.13) because the two data points around 20 µM did not fit the mycorrhizal trend, and may have actually been non-mycorrhizal.

There are a number of reports that mycorrhizae can increase the availability of poorly soluble forms of P, either via solubilization of mineral phosphates, or organically bound phosphate. For example, Marschner and Dell (1994) cited examples where ectomycorrhizae may solubilize calcium phosphates by releasing oxalic acid. Phosphatase production has been

associated with mycorrhizae (eg. Alexander and Hardy 1981), but in such experiments it is difficult to separate the effects of the plant from effects of the fungus. Production of phosphatase by plants occurs in many plant species at low levels of P availability (Li *et al.* 1997), and mycorrhizal inoculation does not necessarily stimulate mineralization of organic matter (Joner and Jakobson 1995). For example, Joner *et al.* (1995) found that mycorrhizal inoculation of cucumber roots had no influence on soil phosphatase activity, but improved P nutrition occurred because the hyphae were able to explore soil where roots had been excluded. Hence mycorrhizal infection increases the effective root surface area or volume of soil that the root system can explore, but the affinity of mycorrhizae for P is likely to be similar to that of the root. Mycorrhizal production of phosphatase enzymes may only occur under some circumstances. Bolan (1991) concluded that increased surface area and exploration of the soil was the most likely cause of mycorrhizal stimulation of growth.

2.3.7 Integration of nutrient uptake and supply principles

Process-based models of nutrient supply and uptake (eg. Barber 1984, Nye and Tinker 1977, Smethurst and Comerford 1993a) are necessarily simplifications of the soil-plant system. These models predict the concentration of a nutrient in solution at an 'average' root surface, using solute transport theory (Section 2.3). Uptake by plant roots is subsequently predicted using Michaelis-Menten kinetic parameters, and uptake over time is calculated by accounting for root growth. Some limitations of these models for predicting phosphate uptake are that (a) they fail to account for changing uptake kinetics with plant nutrient status, stage of growth, and root type (Clarkson 1985), (b) they fail to model the time component of sorption effectively (eg. Barrow 1987), (c) they don't account for chemical changes in the rhizosphere [eg. Saleque and Kirk (1995) found that 80% of P uptake by lowland rice was solubilized after acidification of the rhizosphere], (d) root growth is a major determinant of predicted uptake (Silberbush and Barber 1983), but is difficult to measure and differs with soil type, (e) they don't account for root morphology such as root hairs and mycorrhizal hyphae, and (f)

they assume that soil is spatially homogenous with respect to the distribution of nutrients, water and roots (Rengel 1993). These processes are not described accurately enough for mathematical prediction of their spatial and temporal occurrence.

The method of calculation of the concentration at the root surface used by uptake models also has inherent errors. Models such as that of Oates and Barber (1987) use a numerical method to calculate the concentration at the root surface, which involves iteratively solving simultaneous equation, and requires that inter-root distances held constant. The Smethurst and Comerford (1993a) model uses an analytical method to derive the nutrient concentration at the root surface, which gives a less accurate estimation of the concentration at the root surface, but allows different inter-root distances during the course of the simulation. Despite the limitations of these models, they have been extensively used and often provide accurate predictions of nutrient uptake (eg. Kelly *et al.* 1992, Lu and Miller 1994, Smethurst and Comerford 1993b, Teo *et al.* 1995, Van Rees *et al.* 1990a). Uptake models have also been used to investigate phosphate fertilizer type and placement, and effects of pH on P availability (Barber 1995). The concept of applying these types of models for identification of potential P deficiency is further investigated in Section 2.5.2.

2.4 Management of P fertilizers in eucalypt plantations

2.4.1 Requirement for P during the life-cycle

Miller (1981) identified three nutritional stages in the life-cycle of a *Pinus* plantation. The first stage occurs prior to canopy closure, while the crop is primarily dependent on uptake of nutrients from soil to satisfy the demand for nutrients by an increasing mass of foliage. Nutrient deficiencies are likely to occur if the soil cannot supply enough nutrient to meet demand during this phase. The first phase ends at canopy closure. Leaf area index reaches a maximum (indicating canopy closure) in high growth-rate *E. nitens* and *E. globulus* plantations at approximately 3-4 years (Beadle *et al.* 1995). During the second stage (after

canopy closure), pressure on the soil reserves of nutrients is reduced as nutrients are recycled within the plant and by reabsorption of nutrients after degradation of litter. Stage three occurs many years into the growth of the forest, when nutrient deficiencies can again become more prevalent as the less mobile nutrients accumulate in woody tissue and undecomposed litter.

Eucalypts have a highly efficient system for P recycling within the plant. For example, Crane and Raison (1980) found low P concentrations in *Eucalyptus* wood, compared with *Pinus* wood, and Grove *et al.* (1996) showed that P withdrawal from eucalypt leaves prior to leaf-fall was between 60 and 84% for 6 species of eucalypts. Baker and Attiwill (1985) calculated that only 10% of the P demand of an 80 year old *Eucalyptus obliqua* forest came from soil reserves. A typical rotation length for *E. nitens* and *E. globulus* plantations is between 10 and 40 years, so P deficiency in the second and third stages of Miller (1981) is likely to be less severe than would occur in the first stage. Therefore, the main response to P is likely to occur during the first stage of Miller (1981), ie. during the first 3-4 years prior to canopy closure.

Maximum response to P fertilizer generally occurs during the first year after planting. A significant response has been found to P applied within a few months of planting *E. nitens* plantations, but little response has been observed to P fertilization after the first year at 5 sites in NW Tasmania (G. K. Holz, pers. comm.). Similarly, Birk and Turner (1992) found no response in *E. grandis* following initial applications of P fertilizer. In very P deficient sites, responses to P have been observed after the first year. For example, Cromer *et al.* (1992) found a response to P fertilizer applied to *Eucalyptus deglupta* at 3-4 years of age in Malaysia, and Ward *et al.* (1985) observed a response to fertilizer in 7 year old *Eucalyptus saligna* on a rehabilitated mine site. The latter experiments would probably have been during the first stage of Miller (1981), because canopy closure may not have occurred in those nutrient stressed trees.

A number of experiments with eucalypts have been established in Australia with increasing additions of nutrients over time (Cromer and Williams 1982, Bennett *et al.* 1996, Bennett *et*

al. 1997). For example Bennett *et al.* (1996) established 3 experiments of a factorial N and P design. The treatments were complete after 4 applications, at 2, 9, 14 and 26 months. Because rates of P were confounded with time of application, it was impossible to separate effects of rate and time in those experiments.

In all of the experiments based on increasing nutrient addition over time, growth response to P addition after the first year was minimal, even though higher rates were applied later. Schönau and Herbert (1989), reviewing several P fertilizer timing experiments with eucalypts, also concluded that responses to P fertilization were generally reduced if fertilization was delayed beyond a few months after planting. Supporting this, response to P fertilization after the first year only occurred in 2 of the 13 experiments reviewed above.

The observed lack of response to P fertilizer after the first year in most published experiments may be due to a limitation induced by some factor other than P availability, or it may be that eucalypts increase their ability to acquire P during the first year of growth, such that the contribution from fertilizer applied after the first year is minimal compared to P available at the plant roots.

Regeneration of a native eucalypt forest often occurs after a fire. Fire stimulates seed germination (Attiwill and Leeper 1987) and increases inorganic P in soil solution (Romanya *et al.* 1994). Over time, P in soil solution decreases, as it is occluded in organic matter, and sorbed onto the solid phase. Hence, eucalypt seedlings may have evolved to utilize elevated levels of available P during early stages of growth.

2.4.1.1 Mycorrhizae in eucalypt plantations

In soils of low P availability, mycorrhizal inoculation increases growth and P uptake of eucalypt seedlings, but most evidence is from pot experiments. For example, inoculation of *E. grandis* seedlings with *Pisolithus* isolates increased dry weight (by 100 - 4400%) when grown in a sterilised P-deficient yellow sand (Burgess *et al.* 1994). Inoculation of *E. globulus*

and *E. diversicolor* with different ectomycorrhizal fungi increased growth and P uptake in low-P soils, but in P-sufficient soils mycorrhizal inoculation either depressed growth or had no effect (Burgess *et al.* 1993, Bougher *et al.* 1990). The level of soil P required for maximum growth was not affected by mycorrhizal inoculation (Bougher *et al.* 1990). Growth responses to mycorrhizal inoculation have been obtained in the field in Australia (Thomson *et al.* 1996), and in countries other than Australia (Garbaye *et al.* 1988). However, eucalypts grown in the field in Australia often become infected with 'resident' mycorrhizae (Thomson *et al.* 1996), which reduces the proportion of inoculant mycorrhizae, and masks the effect of mycorrhizal inoculation.

2.4.2 Maintenance of response to first-year fertilization in eucalypts

A sustained growth response to P fertilization at planting in *E. globulus* was found by Cromer and Williams (1982). Growth in the P fertilized treatments was still increasing at a greater rate than that in the control at 9.5 years. Schönau (1984) cited cases where responses to P fertilization of eucalypts at planting were maintained up to reporting date (5 to 12 years after planting). Because rotation lengths of 10 to 20 years are common for pulpwood, growth responses to fertilization in the first year would significantly increase the volume of wood returned at the end of the rotation.

Hence, responses to fertilization at planting are maintained well into the rotation, so indicators of the potential response to fertilization need to be available at planting. Soil analysis was considered an appropriate test, as it could be applied to any site either prior to, or shortly after planting, and the results are available soon after soil collection.

2.5 Improving efficiency of P fertilization in eucalypt plantations

2.5.1 Conventional soil analyses

Traditional tests for soil phosphorus in agriculture and forestry are generally very empirical

in nature, requiring each test to be calibrated for a given crop and soil type. Such tests are based on chemical extractants (see Table 2.7 for a listing of some of the more common analyses), which attempt to provide a measure of P availability by extracting an amount of phosphorus that is equivalent to the reservoir of soil P available to crops (Fox 1981). However, the only pool of P that crops can directly draw from is that in soil solution, replenishment of which occurs at a rate dependent on a number of soil-specific factors. Hence each chemical extractant needs to be empirically calibrated, and may only be useful for that specific crop/soil type system. There are many examples of useful calibrations of such soil tests with crop growth in a given range of soil types (eg. Ballard 1974, Holford 1983, Irving and McLaughlin 1990, Skinner *et al.* 1991, Cox 1994, Wendt 1995, Ashworth and Mrazek 1995).

The extracts in Table 2.7 are approximately in order of increasing strength of extraction. The order is approximate, because the behaviour of each extractant depends on soil type. Weak extractants tend to be indicative of the intensity component of soil P (ie. they correlate with P concentration in soil solution), while the strong extractants are more indicative of the quantity component of soil P (ie. the labile P).

Table 2.7 – Common soil P extractants, in approximately increasing relative strength of extraction.

Extract	Reference	Chemical extract	Extraction time
Calcium chloride	Rayment and Higginson 1992	0.005 M $CaCl_2$	17 h
Olsen	Olsen *et al.* 1954	0.5 M $NaHCO_3$ @ pH 8.5	60 min
Lactate	Egner *et al.* 1960	0.1M Ca lactate + 0.01 M HCl	1.5 h
Bray 1	Bray and Kurtz 1945	0.03 M NH_4F + 0.025 M HCl	40 sec
Acid extractable	Rayment and Higginson 1992	0.005 M H_2SO_4	17 h
Bray 2	Bray and Kurtz 1945	0.03 M NH_4F + 0.1 M HCl	40 sec
Colwell	Colwell 1963	0.5 M $NaHCO_3$ @ pH 8.5	17 hours
Mehlich 1	Mehlich 1953	0.05 M HCl + 0.0125 M H_2SO_4	5 min
Mehlich 3	Mehlich 1984	0.015 M NH_4F + 0.2 M CH_3COOH + 0.25 M NH_4NO_3 + 0.013 M HNO_3	5 min

McLaughlin (1996) cautioned that assessment of plant-available P in forest soils has been less successful than in agricultural soils, due to a number of factors, including poorer nutrient status of forest soils, lower uptake rates by forest tree crops, greater intra- and extra-plant recycling, and greater exploration of the soil resource by tree roots. Very little work has been

done on prediction of P deficiency early in the rotation of eucalypt plantations. Due to low quantities of available P in soil and high levels of organic matter, organic P has been investigated as an indicator of P availability (eg. Turner and Lambert 1985, Adams *et al.* 1989). These indicators are useful for measuring the current status of the forest stand, but may not be very useful for predicting growth at new plantation sites, because of the changes in soil organic matter associated with harvesting and/or other agricultural operations (McLaughlin 1996).

It is accepted that there is no universal test for P for agricultural crops. Both quantity and intensity based analyses are useful in different situations, and it was postulated by Holford (1991, 1997) that successful analyses would need to incorporate both an intensity and a quantity component. Examples of experiments where quantity indices have been well correlated with yield/growth response include: Dalal & Hallsworth (1976), who showed that Colwell P was highly correlated with grain yield of wheat; Jones *et al.* (1995), who found that the Bray P and sorbed P were well correlated with P uptake in a mixture of pasture species, and Holford (1983), who found that the Colwell test was well correlated with growth of white clover in a range of soils. Other researchers have found that indicators of intensity are better for predicting yield, for example, Dear *et al.* (1992) found that concentration of P in solution was best for describing relative yield of subterranean clover at a range of field sites, Moody *et al.* (1983) found that $CaCl_2$ extractable P was better than quantity based indicators for predicting soybean yield, and Ozanne and Shaw (1967) found a good relationship between solution concentration and relative yield of pastures in a number of field experiments. Fox (1981) also provided many examples where intensity-based measurements were excellent at describing relative yield of crops in different soil types, including maize, soybeans, groundnuts and potatoes. Generally, the relationship between stronger extractants and growth is more specific to soil type than with weaker extractants. For example Fox (1981) and Dear *et al.* (1992) found that the relationship between soil test value of a weak extractant was specific to the species under investigation, but not specific to the soil type.

Weak extractants (such as water extractable P and NH_4OAc extractable P) were better correlated with P uptake of *Pinus radiata* in sandy soils (Kadeba and Boyle 1978), and yield of *Pinus taeda* in a range of soils (Tiarks 1982). Both of those correlations were found with short-term pot experiments, which tend to favour the intensity component. Ballard (1974) and Ballard and Pritchett (1975) found that the weaker P extracts such as water extractable P and NH_4OAc extractable P gave high correlations with P fertilizer response of *Pinus radiata* and *Pinus elliotii* in the first year, but growth at 2 and 3 years in the field situation was only correlated with P extracted with stronger analyses (eg. Olsen, Bray and a double acid extract - 0.05 N HCl + 0.025 N H_2SO_4). Hopmans *et al.* (1978) investigated the efficacy of seven quantity-based P analyses for describing height growth of *Pinus radiata* on a range of field sites on a single soil type in Victoria, and found that Bray No. 2 was the only test that gave a significant correlation with growth. There was a trend (but not significant) between response and the Bray No. 1 and Olsen tests in that experiment. Skinner *et al.* (1991) used sequential extractions with a strong extractant (Bray No. 2) to get an indication of the buffer capacity of the soil. Sequential Bray extracts gave good discrimination between sites where a single extract sometimes did not. Any of the common P analyses (eg. those in Table 2.7) could potentially be useful as a predictive test for P availability in eucalypt plantations on specific soil types, but a test that incorporates both intensity and quantity components is more likely to succeed (Holford 1991, 1997).

It is accepted that soil P analyses are soil- and crop-type specific (eg. Holford 1997, Tisdale *et al.* 1993), but soil solution P has proven to be a useful indicator of P deficiency in some crops over a wide range of soil types.

2.5.2 Application of the principles of supply and uptake

Inorganic P in solution is the only form of P that crosses the plasma-membrane of root cells. Hence, it is hypothesised that a test for P based on the concentration of phosphorus in the soil solution would be useful. Knowledge of the principles of nutrient supply and uptake could

potentially be used to determine whether the supply of P to the plant is adequate for optimal growth. Application of these principles, either directly (Fox 1981, Barraclough 1989) or integration via mechanistic models (eg. Nye and Tinker 1977, Barber 1984, Smethurst and Comerford 1993a) may provide a useful test for P deficiency in eucalypt plantations. The mechanistic basis of this approach may make it widely applicable to a range of species and soil-type conditions.

2.6 Research objectives and strategy

The most effective time to fertilize eucalypt plantations with P is at, or soon after, planting (Schönau and Herbert 1989). Some sites are more responsive to P fertilizer than others, but currently there is no test to discriminate between sites for the purposes of P fertilization. Hence, the objective of this project was to identify a useful soil-based indicator of potential P deficiency. The indicator would preferably have wide applicability, both for different soil and crop types.

To answer this objective, the processes limiting P availability in new temperate eucalypt plantations need to be investigated. Both quantity and intensity-based analyses of P availability warranted evaluation. Focus was placed on intensity-based analyses (including the concentration of P in bulk soil solution, and that predicted at the root surface), because of their potential to be well correlated with growth in a wide range of crop and soil-type situations.

3. General Materials and Methods

Materials and methods that were common to more than one experiment are described in this chapter.

3.1 Seed Source and Preparation

Seeds from single open-pollinated seed-orchard trees for both *Eucalyptus nitens* and *E. globulus* were supplied by North Eucalypt Technologies. *Eucalyptus nitens* seeds were graded for size, and those seeds retained on a 20 B.S. (0.84 mm aperture) sieve were used. Average seed weights were 0.5 mg for *E. nitens* and 2 mg for *E. globulus*.

Seeds were surface sterilized by soaking in bleach (3% available chlorine) for 20 minutes. Unless otherwise stated, seeds were osmotically primed for 1-2 weeks in an aerated solution of 2% KNO_3 (Krygsman 1995). Osmotic priming reduces the chronological spread of germination time from about 1 month to less than 1 week. After priming, the seeds were germinated either on moistened cheese-cloth on a stainless steel mesh stand (for solution-based experiments), or in moist sand (soil-based experiments).

3.2 Nutrient Solutions

The nutrient formulation was originally described by Ingestad and Lund (1986) for birch seedlings, but has been used for *E. grandis* by Cromer and Jarvis (1990) and Kirschbaum (1991), and for *E. nitens* by Garnett (1996). The experimental solutions were prepared by dilution of 2 stock solutions (A and B, see Table 3.1) with deionised water (0.24 mL of each stock solution in 10 L of water). The pH in dilute solution was 5.0. Stock solutions were prepared with analytical grade chemicals, and could be stored in the dark for an extended period of time with minimal microbial growth.

Table 3.1 - Chemical composition of stock solutions A and B.

Stock solution A		Stock Solution B	
Compound	Concentration (M)	Compound	Concentration (mM)
NH_4NO_3	2.8	$Ca(NO_3)_2.4H_2O$	170
KNO_3	0.41	$Mg(NO_3)_2.6H_2O$	350
KH_2PO_4	0.1	HNO_3	50
K_2SO_4	0.26	FeEDTA (Na)	7.28
K_2HPO_4	0.32	$MnSO_4.H_2O$	18.5
		H_3BO_3	0.47
		$CuCl_2.2H_2O$	0.46
		$ZnSO_4.7H_2O$	0.07
		$Na_2MoO_4.2H_2O$	87.5

The concentration of P in dilute nutrient solution was 10 μM. The concentration of other nutrients at this P level is shown in Table 3.2. Phosphorus free nutrient solution was prepared by replacement of the KH_2PO_4 and K_2HPO_4 components with KCl. Nutrient solutions were changed weekly.

Table 3.2 - Concentration of nutrients in the diluted solution.

Nutrient	Concentration in solution (μM)	Nutrient	Concentration in solution (μM)
N total	169.7	Fe	0.42
NH_4 N	66.9	Mn	0.17
NO_3 N	102.9	B	0.44
K	39.5	Cu	0.011
P	10.0	Zn	0.011
Ca	4.2	Mo	0.0017
Mg	8.3	Cl	0.022
S	6.2	Na	0.0035

Experimental solutions were aerated with an aquarium pump connected to several 0.9 mm hypodermic needles via rubber and silicon tubing. The culture apparatus was lined with black polyethylene plastic. Polyethylene was used to ensure no solubilization of plastic or plasticisers from the culture apparatus (Hardwick and Cole 1986), and to minimize light penetration into the solution and thereby the risk of algal growth.

3.3 Soil Source and Preparation

3.3.1 Soil

Soils with low P availability were chosen, so that plant growth in unfertilized soils would be limited by P. The main soil used was a Brown Ferrosol (Isbell 1996), with a very high P

buffering capacity. The soil was B-horizon (10-50 cm) material collected from a pit at the 'Sugarloaf' site of North Forest Products, in the Surrey Hills region, 23 km S of Burnie, Tasmania. The site had previously carried a *Pinus radiata* plantation, and had not been fertilized for at least 30 years. The concentration of P in soil solution of the unfertilized soil was approximately 0.1 μM. The soil was brought to Hobart, thoroughly air-dried and mixed before passing through a 5 mm sieve.

3.3.2 Potting of Soil

A weighed quantity of air-dried and sieved (< 5.0 mm) soil was fertilized, and packed to a constant bulk density in 150 mm diameter plastic pots. Soil for each pot was fertilized with basal nutrients (Table 3.3), the rates for which were calculated on a pot surface-area basis. The soil was spread out on a polyethylene sheet, and soluble nutrients (N, K, Ca and Mg) were applied by spraying a combined solution onto the soil. Other nutrients (S, Cu, Zn, Mn, Mo, B and Fe) were applied by spreading a powdered micronutrient fertilizer (Micromax, Sierra Chemical Company, California) evenly over the soil. Phosphorus treatments were applied by including the appropriate amount of KH_2PO_4 in the solutions sprayed onto the soil. All reagents used were analytical grade. Soil and fertilizer were mixed by lifting each end of the polyethylene sheet alternately five times, and then repeating the process another five times at an angle perpendicular to the initial mixing. A small piece of paper-towel was placed in the bottom of each pot to prevent escape of soil from the drainage holes. The soil was watered to a matric suction of 10 kPa, and equilibrated for at least 2 weeks prior to planting.

Table 3.3 - Basal nutrients applied in pot experiments (kg element/ha of pot surface).

Nutrient (form)	Rate	Nutrient (form)	Rate
N (NH_4NO_3)	200	Zn (Micromax™)	3
K (KCl)	60	Mn (Micromax™)	7.5
S (Micromax™)	45	Mo (Micromax™)	0.015
Ca ($CaCl_2$)	30	B (Micromax™)	0.3
Mg ($MgCl_2$)	15	Fe (Micromax™)	36
Cu (Micromax™)	1.5		

3.3.3 Cultural Aspects

Damping off was prevented by spraying the fungicides Fongorid (®Ciba-Geigy) and Previcur (®Schering Agrochemicals) alternately on a weekly basis at the recommended rates, and powdery mildew was prevented with Bayleton (®Bayer Corporation).

Unless otherwise specified, the pots were individually watered 3 times weekly to a predetermined water content (equivalent to 10 kPa matric suction). The pots were randomized weekly within each replicate.

3.4 Soil Analyses

Soil phosphorus was assessed with Calcium chloride, Sorption curve, Colwell, Acid, Bray No. 2, and Paste extracts. Other analyses were loss on ignition, pH and electrical conductivity. Details of each of these analytical methods are described below.

3.4.1 Calcium Chloride Extractable P ($CaCl_2$ P)

$CaCl_2$ extractable P is an indicator of the intensity component of soil P, and was found by Moody *et al.* (1988) to be highly correlated with the concentration of P in soil solution in 26 surface soils from Queensland.

The method used here was similar to Method 9F of Rayment and Higginson (1992). Five grams of air-dry (< 2.0 mm) soil was shaken end-over-end for 17 hours at 25°C with 50 mL of 0.005 M $CaCl_2$. The extract was filtered, and the filtrate analysed for phosphorus. Preliminary analyses indicated that Whatman no. 42 filter papers contaminated the filtrates with P, so 0.45 µm pore-size cellulose acetate filters were used (Lida Manufacturing Corporation, Wisconsin, or Advantec MFS, Inc., California).

Filtered extracts were analysed for orthophosphate on a flow injection analyser (QuikChem 800, Lachat Instruments, Washington, method no. ICNP041A/2A). The $H_2PO_4^-$ ion reacted with ammonium molybdate and antimony potassium tartrate under acidic conditions to form an antimony phospho-molybdate complex. The complex was reduced by ascorbic acid and the intensity of the resultant blue colour was read as an absorbance at 880 nm. The detection limit for this method was less than 0.05 µM P. Results were expressed as a concentration of P in the filtrate (µM).

Moody *et al.* (1988) reported the result of the $CaCl_2$ extract in µg/kg. These data were recalculated to give the concentration in the original extracting solution. The relationship between the concentration of P in the soil solution and the concentration of P in the $CaCl_2$ extracting solution was highly correlated ($R^2 = 0.99$), with a slope of 0.67, ie. the $CaCl_2$ extractable P (expressed in µM) was on average 2/3 of the concentration in soil solution. In soils of high P buffering capacity, the concentration of P in the $CaCl_2$ extract was the same as the concentration in the soil solution (ie. a 1:1 relationship), but these soils did not have a

large influence on the significance of the regression, because solution concentrations were low.

3.4.2 P Sorption Curve

The sorption curve methodology was based on Method 9J of Rayment and Higginson (1992). Five gram samples of air-dry (<2.0 mm) soil were shaken end-over-end at 25°C in 50 mL of 0.01 M $CaCl_2$ extracting solution. Five to seven initial levels of P in the extracting solution were used for each analysis (ranging between 0 μM and 32 000 μM). After shaking for 17 hours, the extracts were filtered through cellulose acetate filters, and the filtrate was analysed for P remaining in solution (method, Section 3.4.1). The relationship between P sorbed by the soil and P remaining in the extract was described by Freundlich, and/or Langmuir equations.

3.4.3 Bicarbonate Extractable P (Colwell P)

The Colwell extraction was based on Method 9B of Rayment and Higginson (1992), but 100 mL extracting bottles were used instead of 250 mL bottles. The filter papers used, and method of analysis of the extract were also different from that presented in Rayment and Higginson (1992). One gram of air-dry soil (< 2.0 mm) was shaken end-over-end at 25°C for 17 hours in 100 mL of 0.5 M $NaHCO_3$ (pH 8.5). The extract was then filtered through cellulose acetate filters, and the filtrate analysed for orthophosphate concentration with a flow injection analyser (QuikChem 800, Lachat Instruments, Wisconsin, QuikChem Method 12-115-01-1-G). The colourimetric principle of the analysis was the same as that in Section 3.4.1, but gaseous CO_2 generated by the reaction was drawn out of solution prior to passing through the cuvette of the spectrophotometer.

3.4.4 Acid Extraction (Acid extractable P)

Acid extractable P was based on Method 9G of Rayment and Higginson (1992), but filter papers and method of analysis were different. Extracts for this analysis were obtained by shaking 1 gram of air-dry soil (<2.0 mm) in 100 mL of 0.005 M sulfuric acid for 17 hours at

25°C. Subsequently, the extract was filtered through cellulose acetate filters. A blue colour was developed in the filtered extracts using the molybdenum blue method of Murphy and Riley (1962), and absorbance at 882 nm was read in a spectrophotometer using a 50 mm path-length cuvette.

3.4.5 Bray No. 2 Extraction (Bray No. 2 P)

The extracting solution for this analysis was initially developed by Bray and Kurtz (1945), and the procedure followed here was the same as used in New Zealand (Blakemore *et al.* 1987). A 2.5 g sample of air-dry soil (<2 mm) was shaken for 40 seconds in 25 mL of extracting solution (0.03 M NH_4F, in 0.1 M HCl). The extract was filtered through cellulose-acetate filters. An aliquot of the filtered extract was diluted five-fold with deionised water, and a blue colour was developed using the colorimetric method of Murphy and Riley (1962). Absorbance of the blue colour was read at 882 nm in a 50 mm path-length cuvette.

3.4.6 Paste Extract (Solution P, P_s)

The method of Smethurst *et al.* (1997) was followed, which involved equilibration of 240 g of field-fresh soil with 60-80 mL of water for 1 hour at room temperature. Following equilibration, the sample was centrifuged at 1300g for 25 minutes, and filtered through cellulose acetate filters. The orthophosphate concentration in the filtered extract was measured on a flow injection analyser, using the same method described in Section 3.4.1.

3.4.7 Loss on Ignition (LOI)

Loss on ignition (LOI) was measured as weight loss from an oven-dried (105°C) sample after 17 hours at 375°C. Organic carbon (OC) was estimated from the relationships between LOI and OC found by Wang *et al.* (1996b).

3.4.8 Electrical Conductivity and pH

Electrical conductivity (EC) and pH were measured on Paste extracts. Electrical conductivity was measured using an electrical conductivity meter (EC meter model 1054, Amber Science, Eugene). Subsequently, pH was measured using a pH meter (Φ34 pH meter, Beckman Instruments, Fullerton).

3.5 Separation of roots from soil

Roots were separated from soil in all pot experiments by spreading the contents of each pot on a polyethylene sheet. The main root systems were carefully removed, and briefly washed with distilled water. 8-10 sub-samples of the remaining soil were bulked and weighed. The bulked subsample was wet-sieved through a 1 mm sieve to separate the remaining root fragments. The total weight (R_T) of root fragments in the original sample was calculated using Equation 3.1.

$$R_T = R_s . \frac{S_T}{S_S}, \qquad \text{Equation 3.1}$$

where R_S was the weight of roots in the subsample, S_T was the total weight of soil and roots, and S_S was the subsample weight of soil and roots.

3.6 Plant Analysis

3.6.1 Leaf measurements

Leaf areas and numbers were assessed by computer image analysis of a scanned photocopy of the leaves.

3.6.2 Root diameter measurements

Roots were spread randomly in a petri dish, and placed under the objective of a travelling microscope. A calibrated graticule in the eyepiece was used to measure the diameter (*d*) of

each root passing through the centre of the eyepiece when the microscope was moved along a random linear transect of the roots in the petri dish. The diameter of the roots was measured to within 0.001 cm. At least 100 diameter measurements were made per root sample.

3.6.3 Root length and surface area estimates

Root lengths were estimated via the line intersect method of Tennant (1975), or were calculated from the average root diameter by assuming that roots of fresh weight (m) were a cylinder of known radius ($r = d/2$). The length (l) was calculated according to Equation 3.2.

$$l = \frac{m / sg}{\pi r^2} \qquad \text{Equation 3.2}$$

Specific gravity (sg) was measured by observing the volume change in a measuring cylinder after addition of a known fresh weight of roots. The specific gravity of seedling *E. nitens* roots was 1.1 g/cm^3. This method of root length estimation was employed more frequently because it was more time efficient than the method of Tennant (1975). Approximate errors for each parameter in Equation 3.2 were the mass of roots (5%), root radius (5%), and specific gravity (10%). Overall error associated with estimation of root length was approximately 20% (Taylor 1982).

3.6.4 Grinding, Wet Digestion, and Analysis

Plant material was wet digested using the sulfuric acid/hydrogen peroxide digestion of Lowther (1980). The material was initially dried at 80°C for at least 48 hours, then ground in a hammer mill. Approximately 0.16 g of redried, ground material was digested in 4 mL of concentrated H_2SO_4, and 2 mL of H_2O_2 (30%, w/v) at 360°C for 30 minutes. After cooling to 150°C, hydrogen peroxide was added dropwise until the solution cleared to a pale yellow colour. The samples were then digested at 360°C for a further 60 minutes, resulting in a clear digestate. The digestate was diluted, and colourimetrically analysed for N (QuikChem method 10-107-06-2E, Lachat Instruments, Wisconsin) and P (QuikChem method 10-115-01-

1D, Lachat Instruments, Wisconsin) on a flow injection analyser (QuikChem 800, Lachat Instruments, Wisconsin).

3.6.5 Dry ashing

Approximately 500 mg of fresh root material was oven dried at 105°C, then slowly heated to 500°C (50 degrees per hour). After 4 hours at 500°C, the samples were left to cool. The resultant white ash was dissolved in 1/20 HCl (v/v).

3.7 Statistical analyses

Non-linear regression analysis was conducted using Systat for Windows, version 5.0 (Systat Inc., USA). The most commonly used non-linear models were the Langmuir (Langmuir 1916), Freundlich (Freundlich 1926) and Mitscherlich (Ratkowsky 1990) functions. Where different models described the same data, tables from David (1938) were used to assess whether the models were significantly different. Other statistical analyses were conducted using Genstat 3.2 for Windows (Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

4. Kinetics of P uptake by *E. nitens* roots

4.1 Introduction

Inorganic P in soil solution is the only form of P that is actively taken by plant roots (Jungk 1991, Marschner 1995), and is therefore a logical component of soil P to investigate as an indicator of P deficient soils. Phosphorus uptake across the plasma membrane is an active process against a steep concentration gradient (Clarkson and Grignon 1991). The concentration range of the high affinity transport mechanism typically encompasses the range of concentrations of P in soil solution of unfertilized soils. Uptake by the high affinity transport mechanism is often described by Michaelis-Menten-like saturation kinetics, which relate influx (I) across the plasma-membrane to concentration in the external solution (x) by the equation:

$$I = \frac{I_{max}(x - C_{min})}{K_m + (x - C_{min})},$$

where I_{max}, K_m and C_{min} are kinetic parameters of uptake (Barber 1984): I_{max} is the maximal rate of influx (ie. when the mechanism is saturated), K_m is the solution concentration at $\frac{1}{2}I_{max}$, and C_{min} is the concentration below which no net uptake occurs. For a graphical representation of these parameters, see Figure 2.9.

Published values for uptake kinetic parameters of several species are shown in Table 2.5. There is considerable variation between species, and it was hypothesised that eucalypt roots may be adapted to low P conditions, because they have evolved on Australian soils, which are renowned for their relatively low P availability (Beckman 1983). The objective of this experiment was to characterise the uptake kinetics of *Eucalyptus nitens* roots. Excised root methodology enabled determination of root uptake ability without interference from the shoot. Excised root methodology was developed by Epstein (1953), and recently validated by Huang *et al.* (1992).

A preliminary experiment (a time-course of uptake) was conducted to establish that steady-state conditions were occurring over the time period of the experiment. The main experiment investigated the concentration dependence of P uptake by roots, and hence the mechanism of P uptake.

4.2 Materials & Methods

4.2.1 Seedling culture

One thousand *E. nitens* seeds were germinated in darkness (method, Section 3.1), then evenly spaced on cheesecloth over a stainless steel mesh screen, with the cheesecloth edges dipped in 0.5 mM $CaSO_4$. After germination, seedling roots were bathed in 10 L of 10 μM P nutrient solution, as described in Section 3.2. The solution was continually aerated, and changed weekly. Plants were grown at a constant temperature of 20°C, and illuminated by a 400 W metal halide lamp, which produced a photosynthetic photon flux density at the leaf surface of 600 $\mu mol/m^2/sec$. After approximately 10 weeks, the seedlings were used for uptake measurements. At this age, the main roots were approximately 100 mm long, and the shoots approximately 50 mm tall. Internal plant reserves of P were depleted by placing them in P-free nutrient solution for 24 hours prior to uptake measurement. Root diameters were measured on unlabelled material (method, Section 3.6.2), and the root length to fresh weight ratio, and surface area to fresh weight ratio were estimated (method, Section 3.6.3).

4.2.2 Uptake Procedure

The method for measuring P uptake kinetics was similar to that of Epstein *et al.* (1963) and Temple-Smith (1973). At 2 minute intervals, approximately 500 mg of roots were excised, quickly blotted, and weighed. Roots were then placed on a circle of nylon gauze (10 cm diameter, 1 mm apertures), which was gathered into a 'tea-bag'. Tea-bags were closed by threading a paper clip through the gathered top; a laminated tag indicating the treatment was attached to the paper clip with 15 cm of nylon line.

The tea-bag of roots was then equilibrated for 20 minutes in 2 L of aerated 0.5 mM $CaSO_4$ solution. After equilibration, tea-bags were placed in 2 L of aerated solution containing P at 25°C for the designated time of the experiment. For the time-course experiment, tea-bags were immersed for 2, 4, 8, 16, 32, 64 or 128 minutes at a fixed concentration of 0.07 µM P. For concentration experiments, the period of immersion was 30 minutes, and the concentrations were 0.01, 0.03, 0.07, 0.1, 0.3, 0.7, 1, 3, 7 or 10 µM of P-32 in solution, adjusted to pH 6.0 with NaOH. The P-32 was supplied as orthophosphate in dilute HCl (Amersham Australia, Pty. Ltd.) and on the delivery date, it had a specific activity of 1120 cpm/nmol P. An aliquot of each solution was taken to determine the initial and final concentrations of P in solution.

After immersion, P-32 was desorbed from the root free space by dipping tea-bags five times in water, and then placing them for 20 minutes in 10 L of unlabelled 10 mM KH_2PO_4/0.5 mM $CaSO_4$ at 4°C.

Roots were then removed from tea-bags, and placed in glass liquid scintillation counting vials (Canberra Packard, Australia), dried and ashed (Section 3.6.5). Each treatment was replicated three times, sequentially using the same experimental solutions. Phosphorus concentration was measured prior to and after each run.

4.2.2.1 Counting Procedure

Cerenkov radiation produced by ß-emission of the decaying P-32 atoms (Läuchli 1969, Parker 1974) was measured with a liquid scintillation counter (Beckman Instruments, California). Raw counts were corrected for background radiation, half-life of P-32, and colour quench (using the sample:channels ratio method of Burns *et al.* 1991). The general liquid scintillation counter settings are shown in Table 4.1, with settings of the individual channels shown in Table 4.2.

Table 4.1 - General settings used on the liquid scintillation counter

Parameter	Setting	Parameter	Setting
Preset time	2.00 min.	Sample:Channels ratio	1:2
RCM	No	Use H# efficiency measure	No
Datacalc	CPM	Unknown replicates	1
Half life	No		

Table 4.2 - Channel settings of the liquid scintillation counter.

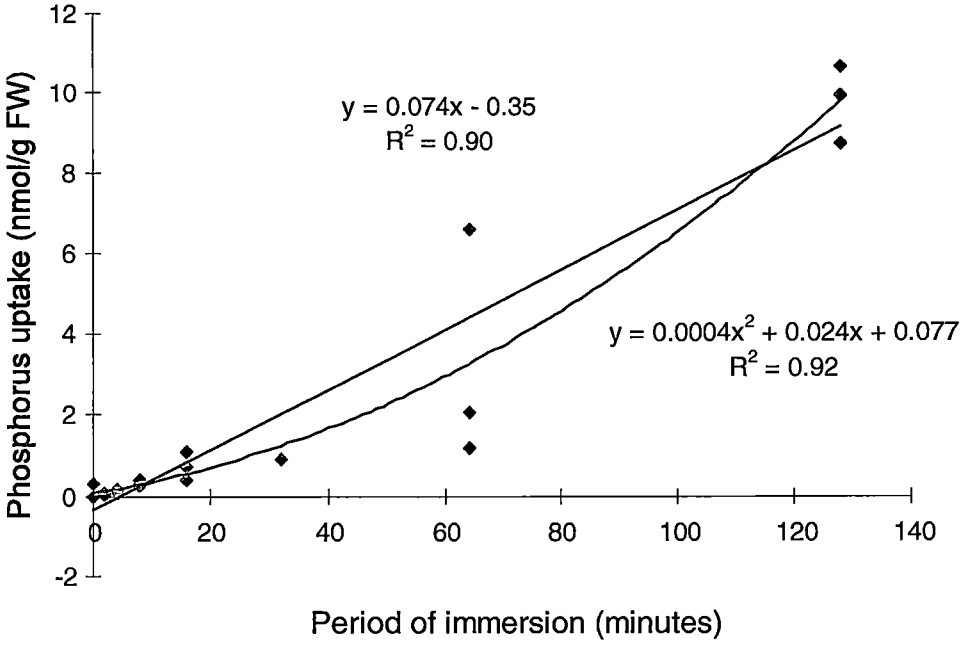
Channel #	Lower limit	Upper limit	2sigma	Background	Bkg 2sigma	LSR
1	0	250	2.00	6.40	35.36	0
2	0	655	2.00	23.40	18.49	0

4.3 Results

4.3.1 Time course of P uptake

The relationship between P uptake and immersion time in the solution (Figure 4.1) followed a linear ($y = 0.074x - 0.29$, $R^2 = 0.90$) or curvilinear ($y = 0.0004x^2 + 0.024x + 0.08$, $R^2 = 0.92$) trend. The models were not significantly different at describing the time-course of uptake. The linear regression intersected the x-axis at 3.96 minutes.

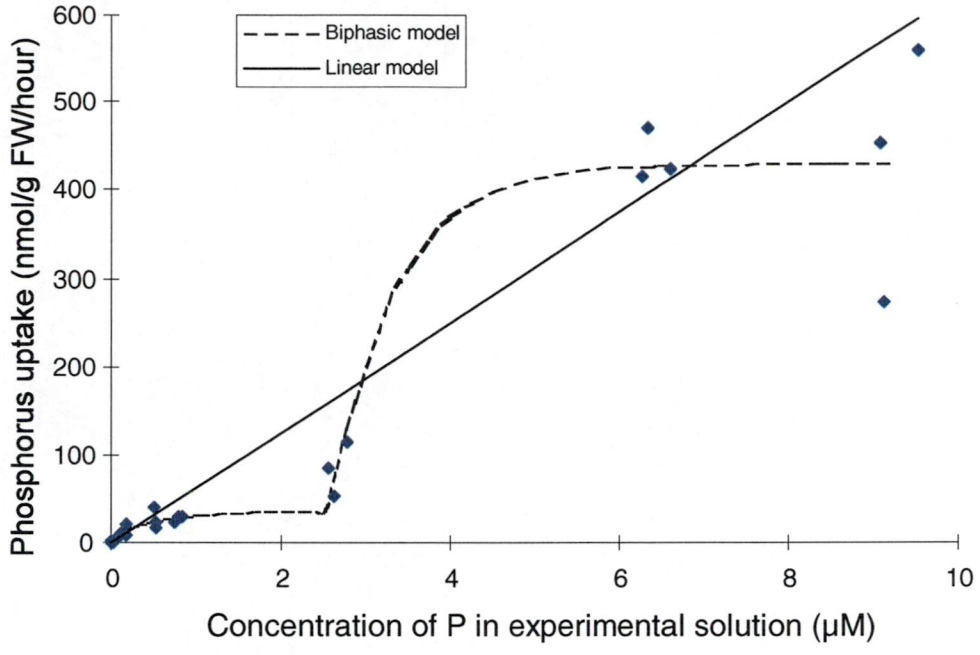
Figure 4.1 - Time course of P-32 uptake by *E. nitens* roots.



4.3.2 Uptake kinetic analysis

A linear regression ($y = 52.522x - 1.443$) explained 94% of the variance in uptake over the entire concentration range of the experiment (0-10 µM, Figure 4.3). However, two separate curvilinear phases in the uptake data were observed. Below 1 µM P in solution, a curve suggesting a high affinity saturable mechanism was well described by a Michaelis-Menten model. Above 1 µM, another mechanism was evident, but kinetic analysis of that mechanism could not be done, due to insufficient data. The dashed line in Figure 4.3 shows a possible 2 phase uptake curve, with a Michaelis-Menten uptake mechanism ($y = \frac{42.545x}{0.374 + x}$) nearing saturation at 1 µM, and an asymptotic regression ($y = 430 - 10000e^{-1.27x}$) above about 2 µM. The high affinity kinetic analysis is shown in more detail in Figure 4.5.

Figure 4.3 - Uptake of P by *E. nitens* roots over the 0-10 µM P concentration range. Possible biphasic and linear models are shown.



Uptake in the high affinity range (below 1 µM) was well described by a Michaelis-Menten model, with kinetic parameters: I_{max} 41.95 (± a standard error of 9.06) nmol/g FW/hour and K_m 0.362 (± 0.178) µM (Figure 4.5). The Michaelis-Menten model described 81.6% of the variance, while a linear regression described 75.0% of the variance (not shown). There was no significant difference between the two models, but the Michaelis-Menten model had a slightly higher R^2 than the linear model, and it allowed a mechanistic interpretation of the nutrient carrier characteristics.

When uptake was expressed on a root length or root surface area basis, K_m remained 0.362 µM, but I_{max} was dependent on the method of expression of uptake. Different units of I_{max} are shown in Table 4.3. Roots at the lowest concentration took up P-32, so C_{min} was less than 0.01 µM.

Figure 4.5 - Phosphorus uptake in the range to 0.01 - 1 μM P.

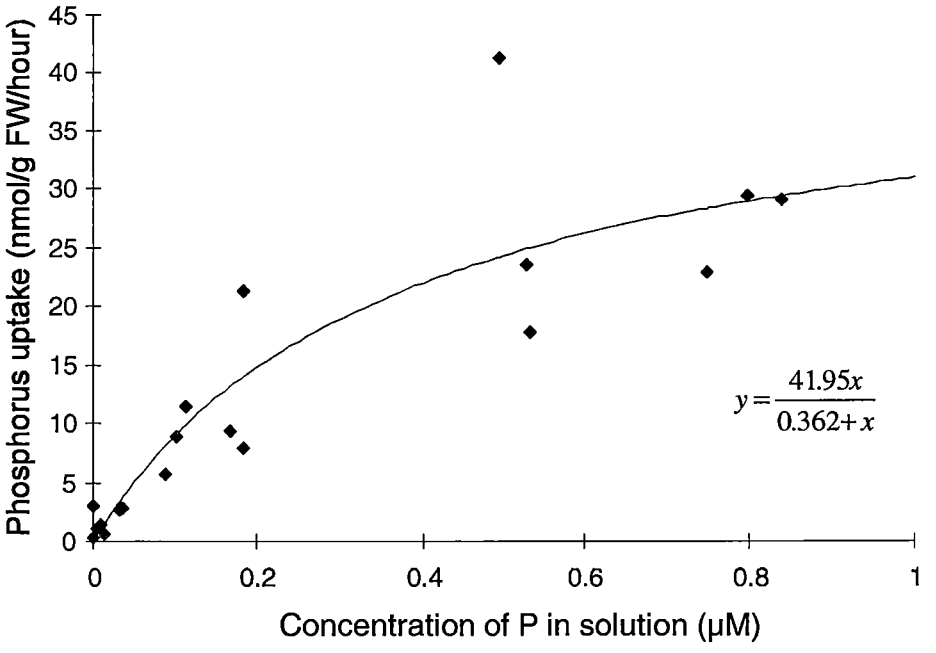


Table 4.3 - Values of I_{max} expressed on a root surface area and root length basis.

Basis for expression	I_{max}	Units
Root fresh weight	41.95	nmol/g FW/hour
Root external surface area	0.35	nmol/cm^2/hour
Root length	0.037	nmol/cm/hour

4.4 Discussion

The time course of uptake was approximately linear. Non-linearity at short immersion times (up to ~10 min) may have occurred because of the lag-time associated with infiltration of P-32 into root free space (ie. cell walls). Only P uptake across the plasma-membrane was

measured, as the method (Section 4.2.2) ensured that phosphorus was desorbed from the free space prior to measuring P taken up. Uptake across the plasma-membrane should occur at a rate independent of time, but only when the AFS is in equilibrium with the external concentration of P. The better fit of the polynomial curve to the time-course of uptake suggested that infiltration of the AFS hindered uptake across the plasma-membrane during the first few minutes of the experiment (up to approximately 10 min.). The intersection of the linear regression with the x-axis gave an estimation of the time required for infiltration of the AFS, and the value of 3.96 minutes for *E. nitens* roots was comparable to the half-time of exchange for phosphate in the free space of barley roots of 2.69 minutes (Lefebvre and Clarkson 1984a). The half-time of exchange for NH_4^+ by spruce root AFS was 28-36 seconds (Kronzucker *et al.* 1995), and by rice seedling root AFS was 1 minute (Wang *et al.* 1993a). Cations have a shorter half-time of exchange, because the permanent negative charge of cell walls electrostatically repels anions, slowing their infiltration into the AFS (Clarkson and Grignon 1991).

Timing of the pretreatment, immersion and rinsing stages of the experiment were controlled to within 10 seconds, so experimental technique was unlikely to be the cause of the variation in uptake. Although seed was harvested from the same mother tree and graded for size, genetic variation would have been high because the pollen used to fertilize each seed was from trees selected for high genetic diversity in a seed orchard (Eldridge *et al.* 1993). Hence, variation in uptake kinetics may have been attributable to genetic heterogeneity of the seedlings.

Epstein and Hagen (1952) suggested that carrier enzymes in the plasma membrane mediate the first phase of uptake, which could be described by Michaelis-Menten enzyme kinetic theory. Some studies have shown that both K_m and I_{max} are influenced by pretreatment concentration (eg. Wang *et al.* 1993b, and Siddiqi *et al.* 1989), but other studies have shown that the change in K_m is relatively insensitive to pretreatment concentration, while I_{max} is

highly dependent on such conditions (see Table 2.5, and Clarkson and Grignon 1991).. Hence, comparison of K_m values is often a useful way to assess differences between species affinity for P, but comparison of uptake or I_{max} is inappropriate, unless the plants are subjected to the same pretreatment conditions.

The concentration of P in soil solution of unfertilized forest soils is typically less than 1 μM (McLaughlin 1996, Smethurst *et al.* 1997), so between 0 and 1 μM is the most important range for characterising the concentration-dependence of uptake. A Michaelis-Menten uptake system appeared to operate up to about 1 μM (Figure 4.3), which would correspond to the high affinity transport system (Clarkson 1985).

The value of K_m was lower than all of those reported in the literature (Table 2.5), and C_{min} was also lower than most values reported in the literature, indicating that *E. nitens* roots had a high affinity for P. The value of I_{max} was also lower than most reported values. Part of the reason for the low I_{max} value may have been the small root radius (0.09 mm), which would have a low AFS:stele volume ratio, and hence a low surface area for absorption. Another explanation for the low I_{max} may have been a plant response to the 10 μM pre-treatment concentration. A number of researchers have found that high pretreatment concentration significantly decreases I_{max} (Lee 1982, Jungk *et al.* 1990, Cogliatti and Santa Maria 1990, Dunlop *et al.* 1997), because of a decrease in the number of P transporters in cell membranes (Clarkson and Grignon 1991).

It has been suggested (for nitrogen, at least), that near-maximal growth of plants can be maintained if the nutrient concentration at the root surface approximates the K_m of the high affinity uptake mechanism (Clarkson 1985, Smart & Bloom 1993). It would be interesting to speculate that the high P affinity of *E. nitens* roots observed in this experiment may be an evolutionary adaptation to grow in Australian soils, which are low in available P (Handreck 1997). The range of K_m values observed for other species indicated that the monocotyledonous plants such as maize and wheat have a lower affinity for P (ie. a higher

K_m), while the species that are the closest to the eucalypts, in terms of affinity for P, were cabbages and lettuces (Temple-Smith and Menary 1977a). Even those plants had a measured K_m several times larger than the K_m found in this experiment.

The K_m value for the eucalypt roots in this experiment was substantially lower than the K_m of *Gigaspora margarita* germ tubes, a vesicular-arbuscular mycorrhizal fungus. The K_m for the high affinity transport system of the fungus was found to be between 1.8 and 3.1 μM, for hyphae that had been pretreated in a 0 μM P growth medium (Thomson *et al.* 1990). Similarly, none of the fungi reviewed by Beever and Burns (1980) had K_m parameters as low as found for *E. nitens* roots in this experiment.

5. Estimation of critical concentration of solution phosphorus for growth of *Eucalyptus grandis* - Analysis of a published experiment.

5.1 Introduction

Sands and Smethurst (1995) showed that N uptake by N-limited birch seedlings in the relative-addition-rate (RAR) experiments of Ingestad and Lund (1979) was not inconsistent with uptake calculated using Michaelis-Menten kinetic theory. Kirschbaum (1991) and Kirschbaum *et al.* (1992) used the RAR technique to investigate the response of *Eucalyptus grandis* to P. *E. grandis* is a sub-tropical eucalypt widely used as a plantation species in Australia and elsewhere. The RAR experiment of Kirschbaum *et al.* (1992) provided the opportunity to estimate the critical concentration and kinetic parameters of *E. grandis*.

The assumptions made by Sands and Smethurst (1995) were that:

a) growth was only limited by the nutrient in question (ie. nitrogen in that experiment),

b) plants were in a steady state during the experiments, with no change in either internal nutrient concentration (n), or root:shoot ratio (r), and

c) nutrient uptake could be described by Michaelis-Menten uptake kinetics.

It was hypothesised that the high affinity uptake mechanism was saturated when relative growth rate (RGR) fell behind RAR. Beyond a critical RAR, nutrient supply exceeded uptake capacity of the plants, so the concentration of N in solution increased over time. Based on these assumptions, Sands and Smethurst (1995) showed that instantaneous seedling relative growth rate (R, /day) was described by their Equation 4:

$$R = \frac{Ur}{n}, \qquad \text{Equation 5.1}$$

where U was the uptake rate. The highest stable RGR (ie. where RAR = RGR), was used to

calculate maximal rate of influx (I_{max}) of the Michaelis-Menten carrier mechanism. Sands and Smethurst (1995) considered two cases: (a) where I_{max} remained constant at each RGR, and (b) where I_{max} increased proportionally with RGR, to a maximum indicated by Equation 5.1. A third case, not considered by Sands and Smethurst (1995), was that I_{max} decreased with increasing RGR (ie. high I_{max} at low RGR, decreasing with increasing RGR). There is considerable published evidence for P that I_{max} increases at low P availability (Lee 1982, Jungk *et al.* 1990, Cogliatti and Santa Maria 1990, Dunlop *et al.* 1997).

Using the calculated I_{max}, and an estimated K_m, the concentration of N in solution (N_S) at each RGR was calculated with Equation 5.2 [Equation 9 of Sands and Smethurst (1995)].

$$N_s = C_{min} + K_m \frac{R_n n / r}{I_{max} - (R_n n / r)} \qquad \text{Equation 5.2}$$

There was good agreement between solution concentrations calculated by Sands and Smethurst (1995), and those observed by Ingestad and Lund (1979), indicating that solution concentration of N could be predicted in RAR experiments using Michaelis-Menten kinetics. The concentration of N in solution at the highest stable RGR could be interpreted as the critical concentration of N.

The purpose of this chapter was to reanalyse the data of Kirschbaum *et al.* (1992) to determine the likely range of values of the critical P concentration and I_{max} of *E. grandis*. The approach of Sands and Smethurst (1995) was extended to include the effect of I_{max} decreasing with RGR.

5.2 Materials & Methods

The original experiment was conducted by Kirschbaum *et al.* (1992). All of the data used in this study was presented in that paper, with the exception of the RGR at each RAR treatment. The latter information was inferred from Figure 1 of Kirschbaum (1991). In summary, seeds of *E. grandis* were germinated and grown in sand for 6 weeks, then transferred to aeroponic

growth units, similar to those used by Ingestad and Lund (1986). While in the growth units, seedlings were supplied for 7 days with a non-limiting supply of a complete nutrient solution, as described in Section 3.2. The internal concentration of P in the seedlings was subsequently reduced by removing the phosphorus component of the nutrient solution for 18 days.

The RAR experiment started after the 18 day pre-treatment, and thereafter, P was added at 5 different RAR's - 0.03, 0.05, 0.08, 0.11, and 0.15 (g/g) /day. Ten plants were destructively harvested from each treatment on nine occasions during the course of the experiment, with the last harvest approximately 120 days after the RAR treatments were imposed.

Concentrations of P that developed in the RGR treatments of Kirschbaum *et al.* (1992) were calculated using Equation 5.2. The nutrient under investigation was P, so the symbols in Equations 5.1 and 5.2 were modified accordingly (Table 5.1).

Table 5.1 - Symbols changed to reflect P uptake in the current experiment.

Parameter	Symbol used by Sands and Smethurst (1995) for N	Symbol used in this study (for P)
Concentration in solution	N_s	P_s
Internal nutrient concentration	n	p
Relative growth rate	R_n	R_p

The values of C_{min} and K_m used in the model were those found for *E. nitens* in the previous experiment, ie. 0 μM for C_{min}, and 0.374 μM for K_m. Three possible scenarios for changing I_{max} were evaluated: constant; proportionally increasing I_{max} with RGR (Sands and Smethurst 1995); and a decreasing relationship between I_{max} and RGR. For the latter treatment, I_{max} was calculated as follows: The value of I_{max} in the experiment of Jungk *et al.* (1990) varied

approximately 5-fold. Hence, it was assumed that I_{max} in the lowest RGR treatment was 5 times that calculated in the maximum RGR treatment, and I_{max} decreased linearly with RGR. In all 3 scenarios, I_{max} at the maximum RGR was that calculated with Equation 5.1.

By assumption (c) of Sands and Smethurst (1995), the concentration in solution at maximal RGR tended to infinity, so the critical concentration of P was calculated at 90% of the maximum RGR.

5.3 Results

Based on the data of Kirschbaum *et al.* (1992), and converting concentrations to a fresh weight basis, a linear relationship was found between the internal concentration of P in seedlings, and relative addition rate (Figure 5.1). The equation for the relationship was $p = 3.26R_P + 0.0188$ ($R^2 = 0.98$).

The root proportion of the dry weight was interpreted from Figure 5 of Kirschbaum *et al.* (1992), and the relationship was linear (Figure 5.2), and described by the equation: $r = -1.09R_P + 0.41$, with an R^2 of 1.00. Relative growth rate was commensurate with RAR for the 0.03, 0.05, and 0.08 /day treatments, but was less than RAR in the 0.11 and 0.15 /day treatments. Hence using assumption (c) of Sands and Smethurst (1995), the Michaelis-Menten uptake system of the roots became saturated at a growth rate between 0.08 and 0.11 /day. The average of these treatments (0.095 /day) was defined as the optimal RGR. Using this relative growth rate, and Equation 4 of Sands and Smethurst (1995), the I_{max} parameter was calculated to be 0.103 mg P/g FW/day (138 nmol/g FW/hour).

Figure 5.1- Relationship between P concentration and RAR in Kirschbaum *et al.* (1992)

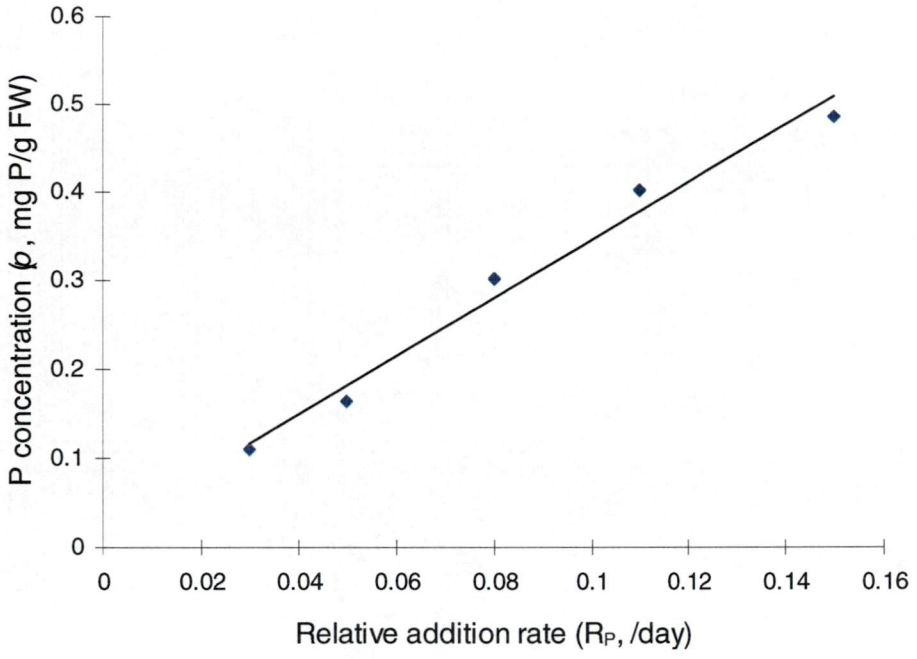
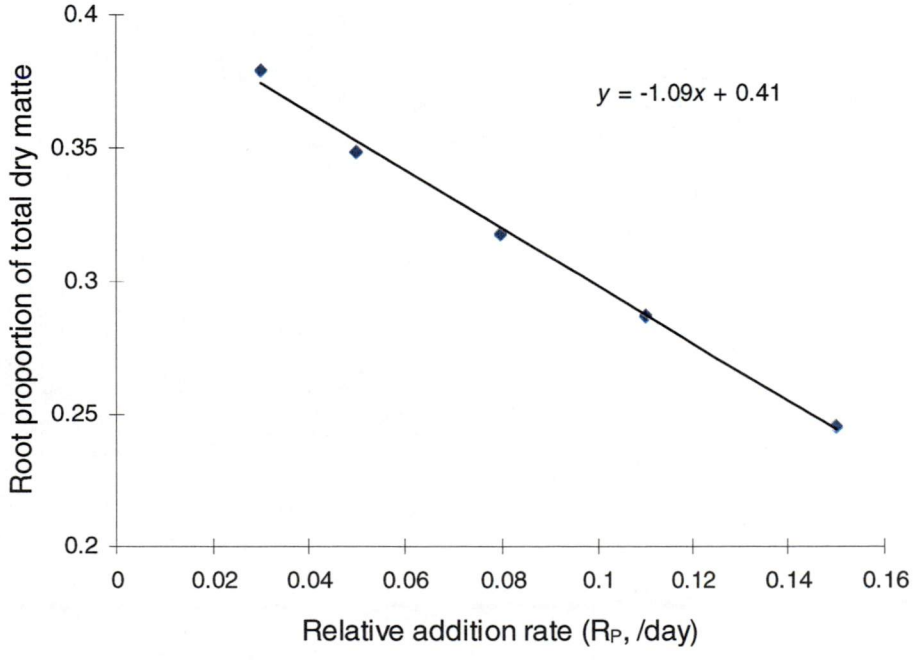


Figure 5.2 - Relationship between root proportion of total dry matter and relative addition rate (from Kirschbaum *et al.* 1992).



The choice of relationship between I_{max} and RGR altered the curvature of the function relating RGR to concentration of P in solution (Figure 5.3), which was reflected in the calculated critical concentrations (Table 5.2). When I_{max} was assumed to change inversely with RGR, the calculated critical concentration was lower.

Figure 5.3 - Relationship between calculated solution concentration and Relative Addition Rate

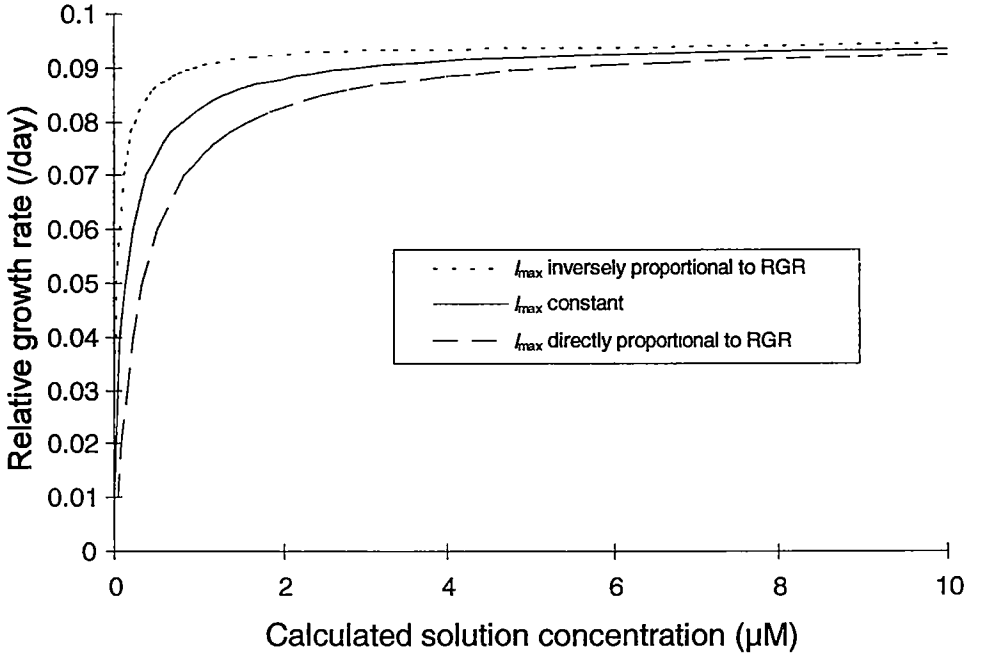


Table 5.2 - Critical concentration of P in solution (ie. at 90% of maximum RGR) with different assumptions of the I_{max} relationship to RGR.

	I_{max}		
	decrease with RGR	constant	increase with RGR
Critical concentration of P (µM)	0.45	1.40	2.64

5.4 Discussion

The estimated I_{max} of 137 nmol/g FW/hour was at the higher end of the range of published values for other species (Table 2.5), and the critical concentration of P in solution (between 0.45 and 2.62 μM P, Table 5.2) was at the lower end of the range of published values of critical concentrations for plants grown in solution culture (Table 2.3). Of the three scenarios for changing I_{max} with RGR, the most likely scenario was that I_{max} was inversely proportional to RGR, because plants compensate for lower internal P status by increasing the potential rate of uptake (Lee 1982, Jungk *et al.* 1990, Cogliatti and Santa Maria 1990, Dunlop *et al.* 1997). The optimal concentration of 0.45 μM under this scenario was low compared with most other species, and indicates that *E. grandis* had a high affinity for-, and a high uptake rate of- P in solution. However, numerous assumptions were required about magnitude of the kinetic parameters (C_{min}, I_{max}, K_m) and the relationship between these parameters and relative growth rate. The requirement for these assumptions reduced the applicability of such an approach until much more is known about the uptake system.

6. Response of *Eucalyptus nitens* and *E. globulus* seedlings to soil P: Glasshouse experiments

6.1 Introduction

Although the concentration of P in soil solution (or in a dilute $CaCl_2$ extract) is not widely used as an indicator of P deficiency, it is well correlated with growth of a number of agricultural crops including soybeans (Moody *et al.* 1983), subterranean clover (Dear *et al.* 1992, Ozanne and Shaw 1967), and other species (Fox 1981). Soil solution P has also been used in combination with buffer capacity to describe P response in wheat (Holford and Cullis 1985) and ryegrass (Holford and Mattingly 1976). Soil solution P has not generally been used to indicate P deficiency in agriculture or forestry, because other chemical extractants are sometimes well correlated with growth (Dalal and Hallsworth 1976), and it has not been assessed for most crops. Where the concentration of P in soil solution was well correlated with plant growth, the relationship was generally not specific to soil type (eg. Fox 1981), whereas the relationship between soil P quantity indicators and plant growth is often highly dependent on soil type (Cox 1994). It was hypothesised that the relationship between *E. nitens* growth response and concentration of P in solution would be less soil-type specific than with P-quantity indicators. If an analysis for P deficiency in *E. nitens* plantations was relatively independent of soil-type, it would require less empirical calibration, and would be more applicable to the wide range of sites currently being planted with eucalypts.

The objective of this chapter was to investigate soil solution-based indicators of growth response to P fertilizer in eucalypts. Firstly *Eucalyptus nitens* seedlings were grown in a highly P buffered brown Ferrosol to investigate the relationship between concentration of P in soil solution, P uptake and growth. The effect of P buffer power on optimum P concentration in solution was investigated in a second pot experiment, with 3 soil types of contrasting P sorption characteristics.

Eucalyptus globulus, a temperate eucalypt in the same taxonomic series as *E. nitens* (Ser. Viminalis, Chippendale 1988), is widely grown in plantations in Australia and overseas. A pot experiment was established to test the hypothesis that both species would respond similarly to P availability.

Responses of young seedlings of *E. nitens* (~ 3 months old) to P were assumed to be indicative of P requirements of 6-to-18-month-old planting stock. Young seedlings were used because they have low internal reserves of P relative to requirement, so they have a high dependence on uptake. After a few months, significant P can be stored within the seedlings (Miller 1989), thereby reducing responsiveness to external P availability compared with young seedlings.

6.2 Materials & Methods

6.2.1 Pot experiment 1: Growth and P uptake of *E. nitens* in relation to the concentration of P in soil solution.

6.2.1.1 Experimental design and set up

The Ferrosol soil described in Section 3.3.1 was chosen for this experiment because of its high P buffer power. It was assumed that soil with a high P buffer power would ensure minimal change in P concentration at the root surface, and maintain the concentration in bulk soil solution at a near constant level for the duration of the experiment.

The soil was prepared as described in Section 3.3.2, except that P was applied as powdered KH_2PO_4 (analytical grade reagent), and basal nutrients were omitted. Six levels of P in soil solution were replicated 3 times in a randomised block design. Planned solution P levels were inferred from a Freundlich sorption curve, which was prepared as described in Section 3.4.2. The planned concentrations and P fertilizer calculated to obtain those concentrations are shown in Table 6.1.

Table 6.1 - Planned concentration of P in solution at each treatment level in the first pot experiment, and P fertilizer calculated to attain those concentrations (inferred from a Freundlich sorption curve).

Treatment	Planned concentration (μM)	P required (mg/kg)	KH_2PO_4 applied (g/pot)
P_0	0.1 (native)	0	0
P_1	0.18	157	0.90
P_2	0.32	325	1.85
P_3	0.56	491	2.81
P_4	1.00	658	3.76
P_5	1.78	824	4.70

6.2.1.2 Plant Establishment and Culture

Eucalyptus nitens seeds were surface sterilized (method, Section 3.1), and ten seeds per pot were sown by placing them individually on the soil surface. A fungicide drench was applied to the pots, which were then covered with black polyethylene to minimize desiccation. Germination occurred over 3 weeks. Pots were randomized, watered, and fungicide was applied following removal of the polyethylene (methods, Section 3.3). In addition to the planted pots, 3 extra pots of the P_2 and P_5 treatments were set up for a separate soil-only harvest.

6.2.1.3 Harvest procedure

The soil-only pots were assessed at 40 days after planting, while the rest of the pots were

harvested at 55 days. Pot contents were spread out on a polyethylene sheet. After removal of visible root systems, an aggregate of 8-10 subsamples of soil was taken for analysis. For soil-only pots, $CaCl_2$ P, and water content were determined. For planted pots, plant tops were removed by cutting as close as practicable to the soil surface. Any soil around the base of the stem was brushed off. Shoot height, stem diameter and leaf area were measured. Plant material was then bulked per pot and dried in paper bags at 80°C. Dry weights and tissue N and P concentrations were measured on a pot-basis. Roots were extracted from the soil as described in Section 3.5.1. Root fresh weight, dry weight, and diameter were measured (methods, Section 3.6).

6.2.1.4 Calculation of competition between roots for P

To assess the degree of competition between the roots, the depletion zone of P around the root was calculated according to Equation 6.1:

$$R_D = 2\sqrt{D_e.t} + R_0, \qquad \text{Equation 6.1}$$

(Nye and Tinker 1977), where R_D is the radius of the depletion zone around the root, D_e is effective diffusion coefficient of P through the soil, t is time, and R_0 is root radius. The effective diffusion coefficient (D_e) was calculated using Equation 6.2:

$$D_e = \frac{D_l.f.\theta_v}{b}, \qquad \text{Equation 6.2}$$

where D_l is the diffusion coefficient in liquid, θ is volumetric water content of the soil, and f is an impedance factor (a measure of the tortuosity of the diffusion path in soil), which was related to soil moisture content via Equation 6.3 (Nye and Tinker 1977).

$$f = \sqrt{\theta_v}, \qquad \text{Equation 6.3}$$

Buffer power (b), was calculated using Equation 6.4 (Van Rees *et al.* 1990b).

$$b = \theta_v + \rho K_d ,$$ Equation 6.4

where ρ is the bulk density of the soil and K_d is the partitioning coefficient between the liquid and solid phases of soil P (calculated via an adsorption isotherm). Equations 6.1, 6.2, and 6.3, and the parameters in Table 6.2 were used in a modified Smethurst and Comerford (1993a) model (COMP82, Section 8.2.1) to calculate depletion zone width. Root growth parameters in Table 6.3 were used to calculate volume of soil within the depletion zone. The curvature coefficient of root growth (ie. *b* parameter) was set at 1.05, which was similar to the curvature of shoot growth in pot experiment 2. The *a* and *c* parameters were derived to give the measured initial and final root length densities. To support the assumption that shoot growth could be used to model root growth, Shepherd and Sa-ardavut (1984) found that root:shoot weight ratio remained close to constant during development of *Eucalyptus camaldulensis* seedlings up to the 14 leaf-pair stage.

Table 6.2 - Parameters used in each treatment of pot experiment 1 to calculate depletion zone volume around roots.

Parameter	Value	Parameter	Value
D_l (cm^2/sec)	8.9 x 10^{-6}	Freund. a^A	7385
θ_v (g water/g soil)	0.4199	Freund. b^A	1.39
root radius (cm)	0.004671	ρ (g/cm^3)	0.8

^Freundlich equation: $y = a.x^{1/b}$, where y was P sorbed to the solid phase (µg/g soil), x was the concentration of P in solution (µg/mL), and a and b were fitted parameters.

Table 6.3 - Root growth parameters used for depletion zone calculations.

Treatment	Root growth parameters[A]		
	a	*b*	*c*
P_0	2.54×10^{-3}	1.05	-2.53×10^{-3}
P_1	4.91×10^{-3}	1.05	-4.91×10^{-3}
P_2	9.31×10^{-3}	1.05	-9.31×10^{-3}
P_3	8.74×10^{-3}	1.05	-8.73×10^{-3}
P_4	8.51×10^{-3}	1.05	-8.51×10^{-3}
P_5	9.45×10^{-3}	1.05	-9.44×10^{-3}

[A] Root growth equation: $y = ab^x + c$, where y was the root length density (cm/cm^3), x is time (d), and a, b and c were fitted parameters.

Specific absorption rate (SAR) of roots is a useful indicator of the effectiveness of roots for acquiring phosphate (Barrow 1977), and was calculated for *E. nitens* roots in pot experiments 1 and 2 using Equation 6.5 (from Barrow 1977).

$$SAR = \frac{1}{W_r} \cdot \frac{dW_p}{dt} \qquad \text{Equation 6.5}$$

where W_r was the root weight (g), and W_p was the weight of P (g) taken up at time t. SAR was calculated on a daily basis, and presented as an average for the experiment.

6.2.2 Pot experiment 2: Growth and P uptake of *E. nitens* in soils of different P-sorption characteristics.

6.2.2.1 Experimental design and set up

Four soils low in available P, and of contrasting P buffer power were sampled from 1 Victorian and 3 Tasmanian plantations (Table 6.4). Hereafter, the soils are referred to by their texture. The Ferrosol soil from the first pot experiment was also included (Clay loam 2). Sorption curves for each soil were prepared as described in Section 3.4.2.

Table 6.4 - Characteristics of the soils used in the second pot experiment

Texture	Silty clay loam	Clay loam 1	Sand	Clay loam 2
Collection location	Nabowla (Tas.)	Florentine valley (Tas.)	Gippsland (Vic.)	Surrey Hills (Tas.)
Isbell (1996) classification	Brown Kurosol	Brown Kurosol	Arenic Rudosol	Brown Ferrosol
Depth (cm)	0-15	0-10	0-10	10-50
Bulk Density	0.8	0.7	1.4	0.8
pH (1:5)	5.2	5.0	n/d	4.9
LOI (%)	5.1	11.9	n/d	15
EPC[A] (µM)	0.21	0.06	0.16	0.10
Buffer capacity at EPC[A]	919	2989	724	62197

[A]Calculated from a sorption curve with units of solid phase P (µg/g) on the y axis, and liquid phase P (µg/mL) on the x-axis. To be consistent with concentrations quoted later, EPC was converted from µg/mL to µM.

Four levels of P in soil solution were established in each soil. One pot per treatment was harvested for three sequential harvests, followed by 3 pots per treatment at the fourth and final harvest. Pots were arranged in a randomised block design in a glasshouse. The planned levels of P in soil solution were greater than those required (Table 6.5), to account for differences between concentrations inferred from a short-term sorption curve and those observed in the first pot experiment. Soils were prepared as described in Section 3.3.2.

Table 6.5 - List of treatments, and planned concentrations of P in the soil solution

Treatment:	P_0	P_1	P_2	P_3
Expected concentration (μM):	native	0.5	1	2

Concentrations of $CaCl_2$ P in the Ferrosol soil remained very low (less than 0.06 μM) in all treatments, and the measured concentrations were highly variable because they were near the detection limit for P. Results for the Ferrosol in this experiment were not included in further analyses, and were substituted by those from the same Ferrosol used in the first pot experiment.

6.2.2.2 Plant establishment and culture

Seedlings were germinated and grown in low nutrient sand for approximately 1 month, prior to transplanting into pots. Eight seedlings per pot were transplanted, when root length was approximately 6 cm.

Twenty-five percent of seedlings in the clay loam 1 soil showed signs of herbicide (possibly atrazine) damage. The affected plants grew slowly, were chlorotic and malformed, and were excluded from further consideration. Enough healthy plants remained to observe trends in P response. This soil was collected away from the area normally sprayed, but it was possible

that a knapsack sprayer had been emptied over the area several years previously.

6.2.2.3 Harvest procedure

The first three harvests were at 30, 47 and 68 days after transplanting, and the fourth and final harvest was at 82 days after transplanting. The soil was analysed for water content, $CaCl_2$ P and Colwell P (Section 3.4). Plant tops were removed by cutting as close to the soil surface as practicable, and any soil around the base of the shoot was brushed off. Height, diameter (at the base), and leaf area were measured on fresh material. Roots were extracted from the soil, and fresh weights, dry weights, and root diameters were measured. Shoot and root dry weights were measured after drying at 80°C for 48 hours. Roots were extracted from 1 pot per treatment at each harvest.

Table 6.6 - Parameters used in each treatment of pot experiment 2 to calculate depletion zone volumes around roots.

Parameter	Silty clay loam	Clay loam 1	Sand
D_l (cm^2/sec)	8.9×10^{-6}	8.9×10^{-6}	8.9×10^{-6}
θ_v (g water/g soil)	0.43	0.53	0.045
Freund. a^A	20.452	1570.2	13.074
Freund. b^A	5.282	1.062	3.746
ρ (g/cm^3)	0.8	0.7	1.4

^Freundlich equation: $y = a.x^{1/b}$, where y was P sorbed to the solid phase (µg/g soil), x was the concentration of P in solution (µg/mL), and a and b were fitted constants for each soil.

Table 6.7 - Root growth Parameters used to calculate volume of soil in depletion zone around roots in the second pot experiment.

Soil	Treatment	Root growth parameters		
		a	*b*	*c*
Silty clay loam	P_0	2.0×10^{-3}	1.05	2.44×10^{-3}
	P_1	8.2×10^{-4}	1.07	3.62×10^{-3}
	P_2	5.5×10^{-4}	1.08	3.89×10^{-3}
	P_3	8.7×10^{-4}	1.07	3.58×10^{-3}
Clay loam 1	P_0	2.8×10^{-3}	1.02	-2.37×10^{-2}
	P_1	1.9×10^{-3}	1.04	-1.41×10^{-2}
	P_2	4.9×10^{-5}	1.13	4.40×10^{-3}
	P_3	5.1×10^{-5}	1.13	4.40×10^{-3}
Sand	P_0	3.9×10^{-3}	1.04	-3.48×10^{-2}
	P_1	3.6×10^{-3}	1.02	-3.10×10^{-2}
	P_2	6.5×10^{-4}	1.08	3.79×10^{-3}
	P_3	6.9×10^{-4}	1.08	3.76×10^{-3}

^ Root growth equation: $y = ab^x + c$, where y was root length density (cm/cm^3), x was time (d), and a, b and c were fitted parameters.

Depletion zones, and volume of soil around roots in each pot were calculated using the modified Smethurst and Comerford (1993a) model (COMP82), and the parameters in Table

6.6, and Table 6.7.

The curvature of root growth (*b* coefficient) was inferred from the curvature of shoot growth in each treatment, and the *a* and *c* parameters were derived to fit root length density at the beginning and end of the experiment. Solution concentration and root diameters were different for each treatment (see Results).

Relative shoot dry weight in pot experiments was determined as dry weight in control as a percentage of predicted maximum dry weight (Equation 6.6).

$$\text{Relative shoot DW (\%)} = \frac{\text{DW in control treatment}}{\text{predicted maximum DW}} \times \frac{100}{1} \qquad \text{Equation 6.6}$$

6.2.3 Pot experiment 3: Comparison between *E. nitens* and *E. globulus* growth responses to soil P.

6.2.3.1 Experimental design and set up

A new bulk sample of the Ferrosol soil used in the first pot experiment was used in this experiment. Two species were treated with six levels of P in solution (Table 6.8), replicated six times. The planned levels of P in solution were inferred from a Freundlich sorption curve. Planned concentrations were about double those required, to account for the difference between concentrations predicted from a Freundlich isotherm, and concentrations that developed in previous pot experiments. The soil was prepared as described in Section 3.3.

Table 6.8 - Planned concentration of P in solution at each treatment level in the third pot experiment, and P fertilizer required to obtain those concentrations (inferred from a Freundlich sorption curve).

Treatment	Planned Concentration (μM)	P Required (g P/pot)	KH_2PO_4 applied (g/pot)
P_0	0.1	0	0
P_1	0.16	0.05	0.23
P_2	0.25	0.19	0.85
P_3	0.40	0.36	1.59
P_4	0.63	0.56	2.46
P_5	1	0.87	3.84

6.2.3.2 Plant establishment and culture

Seeds of *E. nitens* and *E. globulus* (700 each) were osmotically primed, germinated, and grown in nutrient-poor sand for 1 month prior to transplanting (Section 3.1). Poor germination of *E. globulus* seedlings gave only enough for 1 seedling per pot, whereas 4 *E. nitens* seedlings per pot were planted. Pots were set up as described in section 3.3. Only 3 replications of the P_4 and P_5 treatments were set up. Overhead sprinklers were programmed to water pots for 3 minutes at 4 times each day. This régime provided a sufficient quantity of water for plant growth, but minimised leaching losses.

6.2.3.3 Harvest procedure

Plants were harvested 70 days after transplanting to measure shoot dry weight. Liverwort

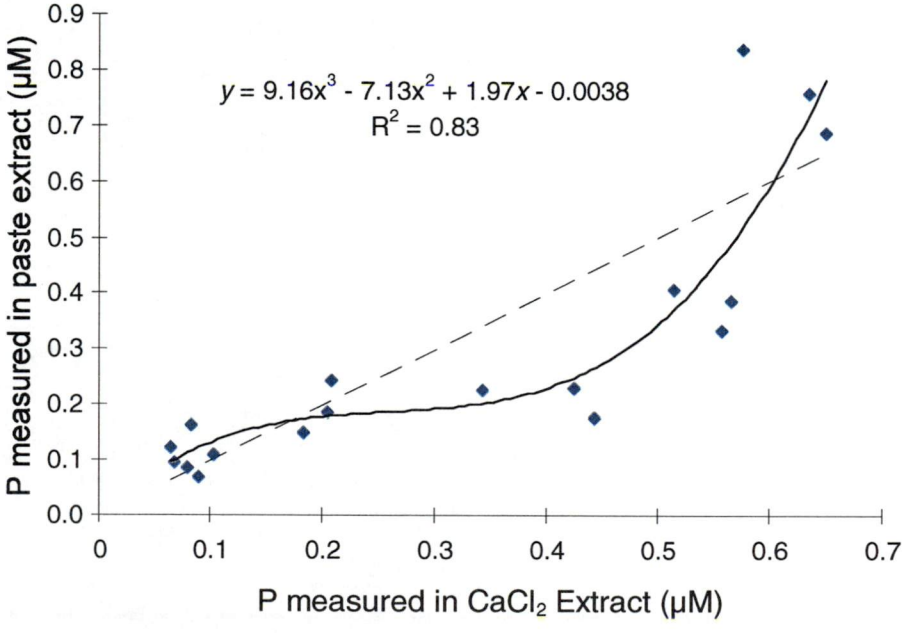
growth was encouraged by regular watering, so percentage liverwort coverage was also assessed in each pot.

6.3 Results

6.3.1 Pot experiment 1: Growth and P uptake of *E. nitens* in relation to the concentration of P in soil solution.

The $CaCl_2$ extract was assessed for its ability to measure solution P by comparing the results with paste P (P_s). The relationship between P_s and $CaCl_2$ P in the Ferrosol was described by the polynomial regression: $y = 9.16x^3 - 7.13x^2 + 1.97x - 0.0038$ (Figure 6.1), which accounted for 83% of the variation between P_s and $CaCl_2$ P over the range 0.05 to 0.65 µM $CaCl_2$ P. The $CaCl_2$ extract was used to indicate concentration of P in soil solution for further analyses.

Figure 6.1 - Relationship between $CaCl_2$ P and P_s for a highly P buffered Ferrosol. Dashed line represents 1:1 relationship.



Phosphorus sorption characteristics of the Ferrosol (0 - 20 000 µM range) were described by

a Freundlich model ($y = 718.53x^{1/4.707}$, $R^2 = 0.983$, Figure 6.2), but the range of interest for plant growth was between 0 and 2 μM (Figure 6.3). The overall Freundlich function poorly described sorption in the 0 - 2 μM range, so a second Freundlich model ($y = 606.019x^{1/1.390}$, $R^2 = 0.94$) was derived to fit the data in that range. The second Freundlich model was used to predict P fertilizer required to obtain the planned soil solution concentrations.

Figure 6.2 - Phosphorus sorption curve in the 0 - 20 000 µM solution P range.

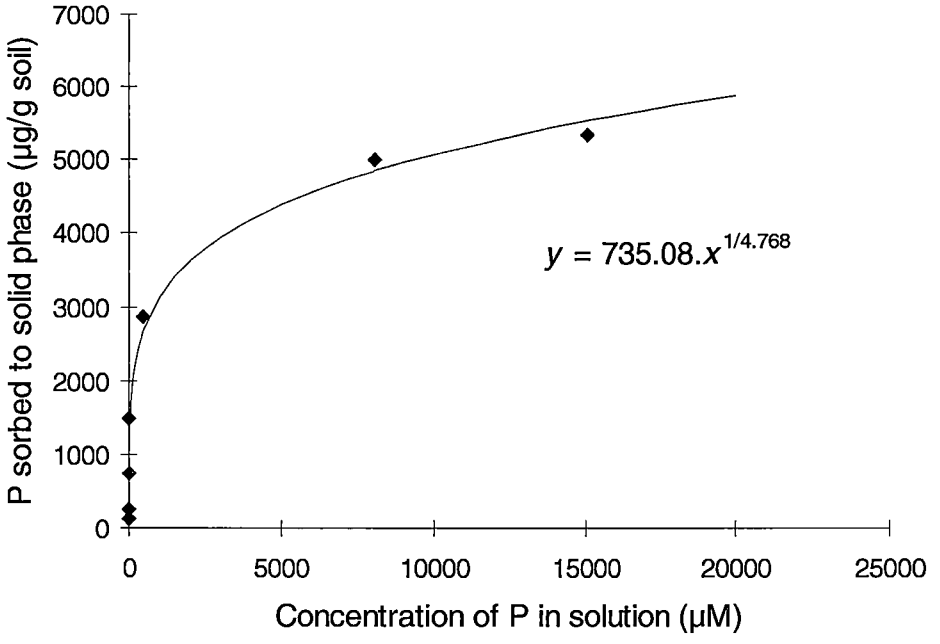
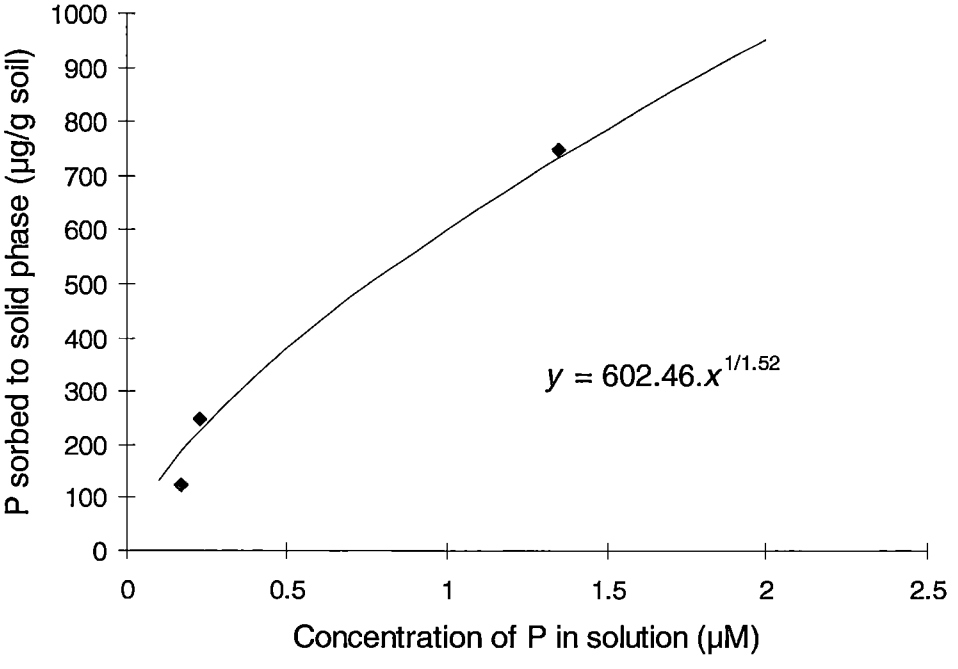
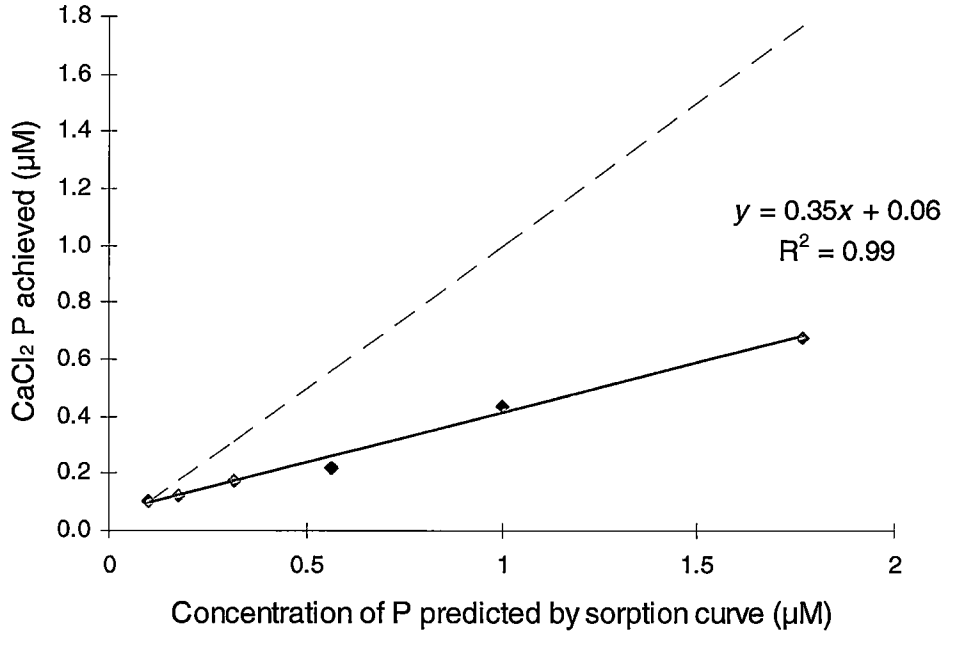


Figure 6.3 - Sorption curve in the range of concentrations of interest (0 - 2 µM P)



Actual concentrations of P achieved in soil solution (as measured by the $CaCl_2$ extract) were 35% of those desired (Equation: $y = 0.35x + 0.06$, $R^2 = 0.99$, Figure 6.4). The determination coefficient (R^2) of 0.99 indicated a highly correlated relationship between planned and achieved concentrations.

Figure 6.4 - Relationship between planned and achieved concentrations of P in soil solution. The dashed line represents a 1:1 ratio.



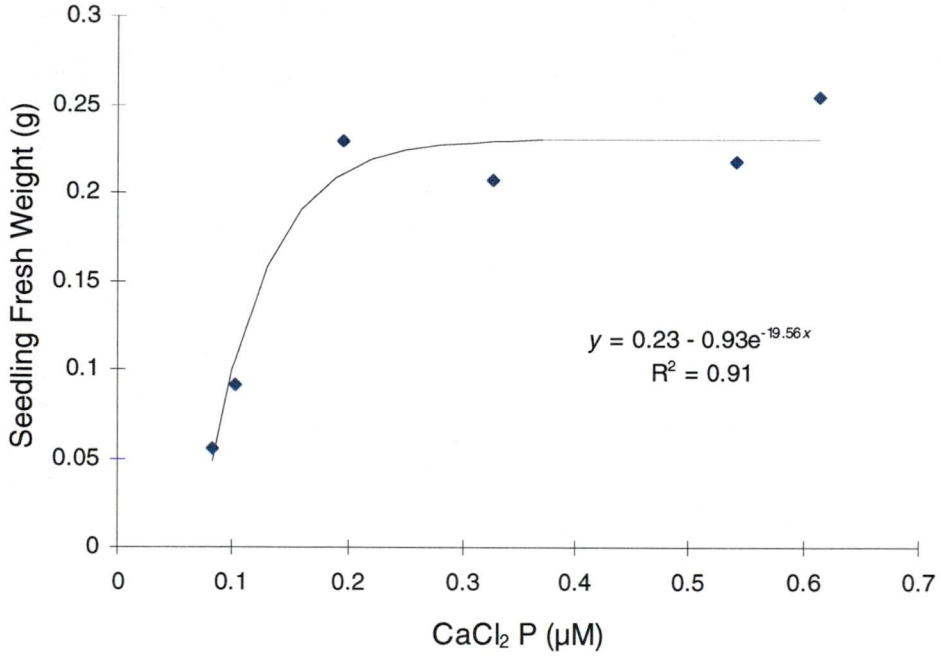
Relative growth rates of the *E. nitens* seedlings over the 64 days of the experiment ranged from 0.048 - 0.064 /day (Table 6.9), and were similar in the last four treatments.

Table 6.9 - Relative growth rates observed in the pot experiment

Treatment:	P_0	P_1	P_2	P_3	P_4	P_5
RGR[A] (/day):	0.048	0.052	0.063	0.062	0.062	0.065

[A]Relative growth rate calculated using the equation: $\text{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$, where W_1 and W_2 were initial and final weights, and t_1 and t_2 were initial and final time (Ingestad 1988).

Figure 6.5 - Seedling fresh weight response to concentration of P in $CaCl_2$ extract.

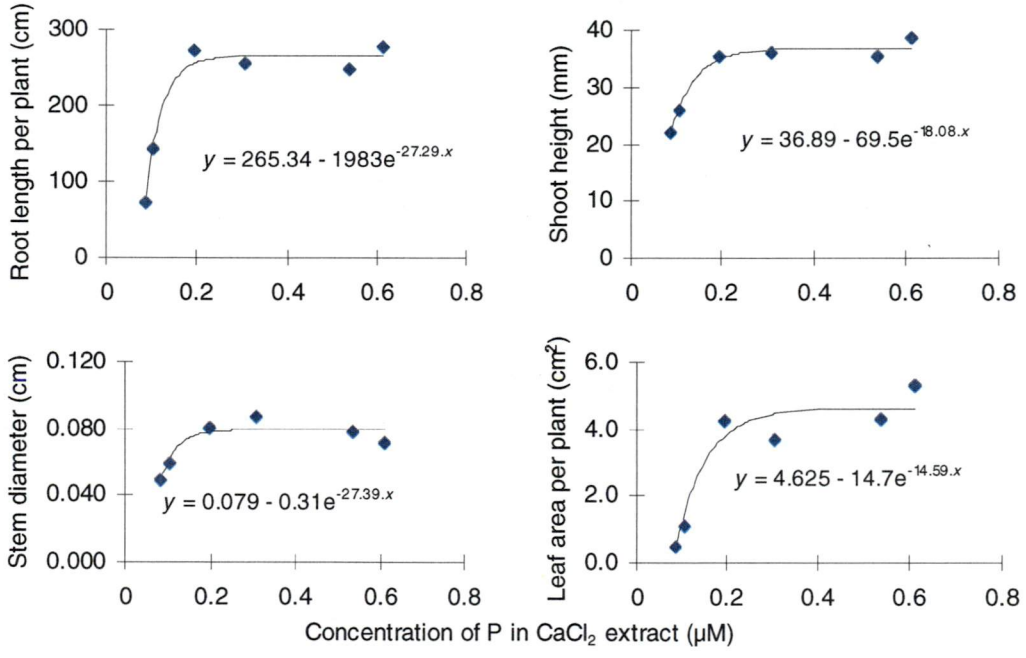


The relationship between growth and $CaCl_2$ P was asymptotic (Figure 6.5), and well described by a Mitscherlich function ($y = 0.231 - 0.925e^{-19.56.x}$, $R^2 = 0.91$). Analysis of variance of the square-root transformed data showed that growth in the first two treatments was significantly lower than the remaining four, which were not significantly different from each other. The asymptote of the Mitscherlich function (ie. maximum seedling fresh weight) was 0.231 g, and 90% of this fresh weight was achieved at a $CaCl_2$ P concentration of 0.19

μM (equivalent to 0.18 μM P in the soil solution).

Similar relationships were also observed for the relationship between $CaCl_2$ P and other growth traits, including root length, seedling height, stem diameter and leaf area (Figure 6.6). Critical concentrations of $CaCl_2$ P (ie. for 90% of maximum growth) for each of the traits were calculated to be 0.16, 0.16, 0.13, 0.24 μM, for root length, seedling height, stem diameter and leaf area, respectively. Differences between the traits were due to the sensitivity of individual parameters to P deficiency.

Figure 6.6 - Root length, shoot height, stem diameter and leaf area relationship with $CaCl_2$ P.

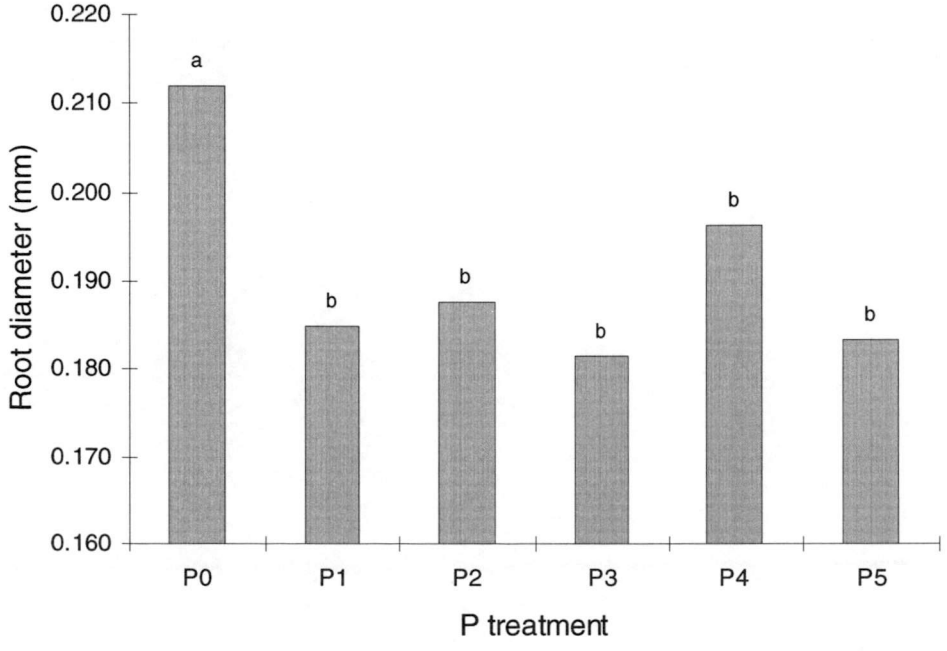


To estimate the sensitivity of each growth attribute to P deficiency, growth in the control treatment was expressed as a percentage of predicted maximum (Table 6.10). Attributes more sensitive to P deficiency had a lower percentage growth parameter in the unfertilized soil. Leaf area was most sensitive to P deficiency, followed by seedling weight and root length. Shoot height and stem diameter were poor indicators of P deficiency.

Table 6.10 - Calculated sensitivity of growth parameters to P deficiency (in order of decreasing sensitivity).

Growth parameter	minimum value	predicted maximum	% of max. in unfertilized soil
leaf area (cm^2)	0.52	4.63	11.3%
Seedling FW (g)	0.056	0.23	24.3%
Root length (cm)	74.35	265.34	28.0%
Shoot height (mm)	22.15	36.89	60.0%
Stem diam. (mm)	0.50	0.79	62.7%

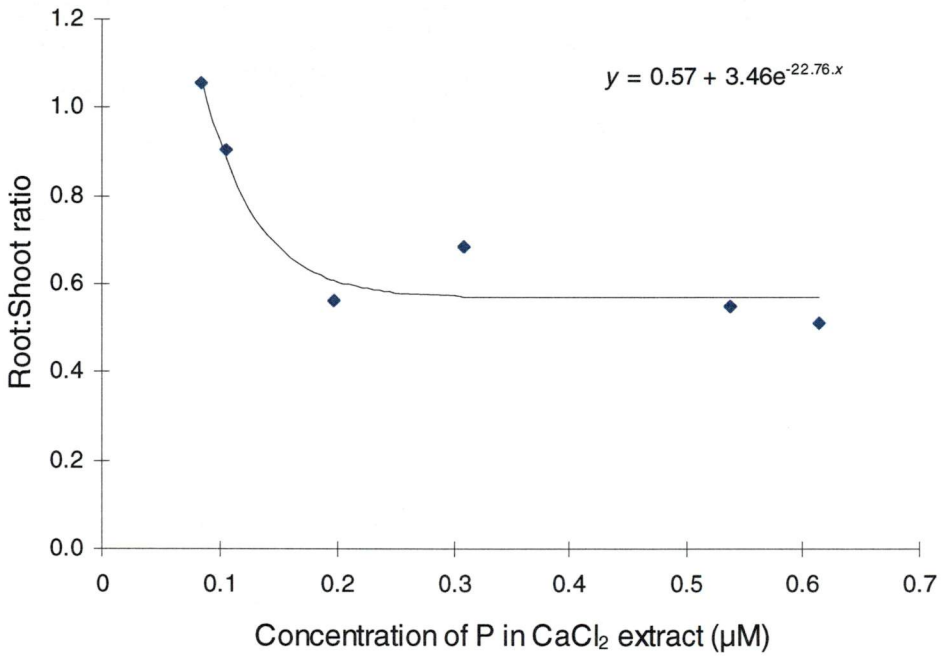
Figure 6.7 - Root diameter variation with treatment. Treatments with the same letter were not significantly different (P = 0.05).



Average root diameters in the control treatment were significantly larger than in the other treatments (Figure 6.7), but there was no significant difference in average root diameter between the P-fertilized treatments.

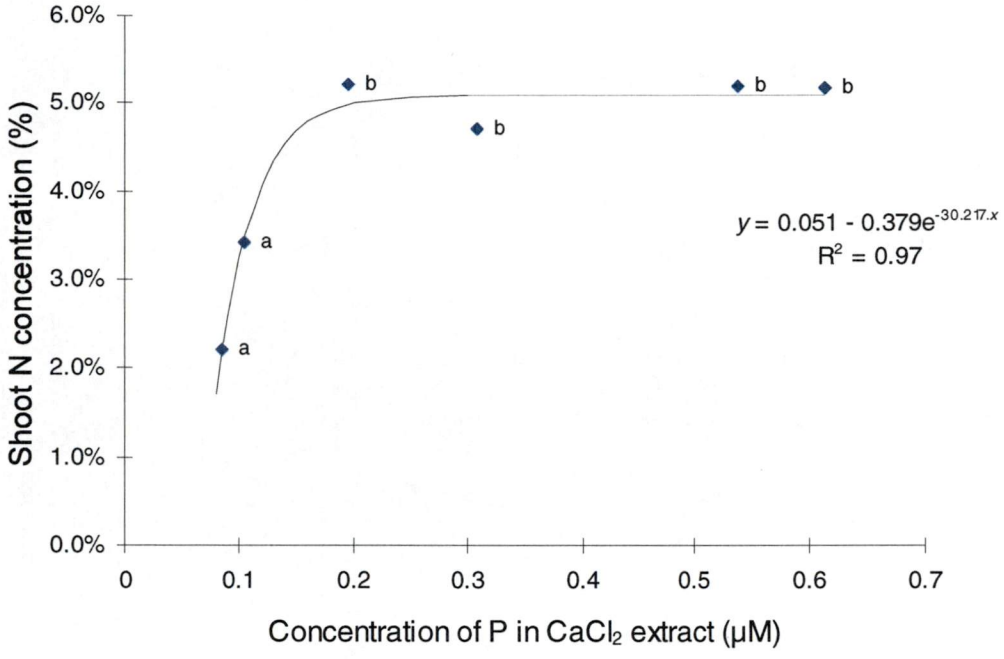
Plants in higher P treatments allocated proportionally more biomass to shoot growth (Figure 6.8). The root system made up slightly more than 50% of the biomass at the lowest P treatment, while 30-40% of the biomass was allocated to roots in the higher P treatments.

Figure 6.8 - Root:shoot fresh weight ratio variation with $CaCl_2$ P.



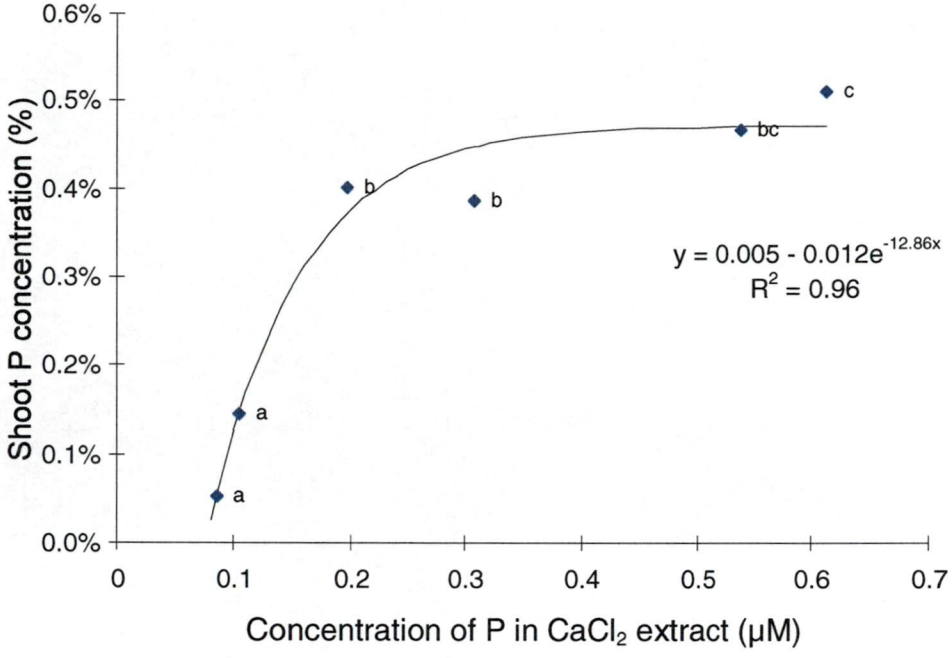
A Mitscherlich function ($y = 0.051\text{-}0.379e^{-30.22.x}$, $R^2 = 0.96$) described the response of shoot N concentration to P in solution (Figure 6.9). The asymptote of the Mitscherlich function occurred at 5.09% N, and 90% of maximum tissue N concentration occurred at a $CaCl_2$ P of 0.168 µM P. Analysis of variance showed that tissue N concentrations in the first two treatments were significantly different to those in the last four treatments.

Figure 6.9 - Shoot N concentration relationship with $CaCl_2$ P. Treatments with the same letter were not significantly different (P = 0.05).



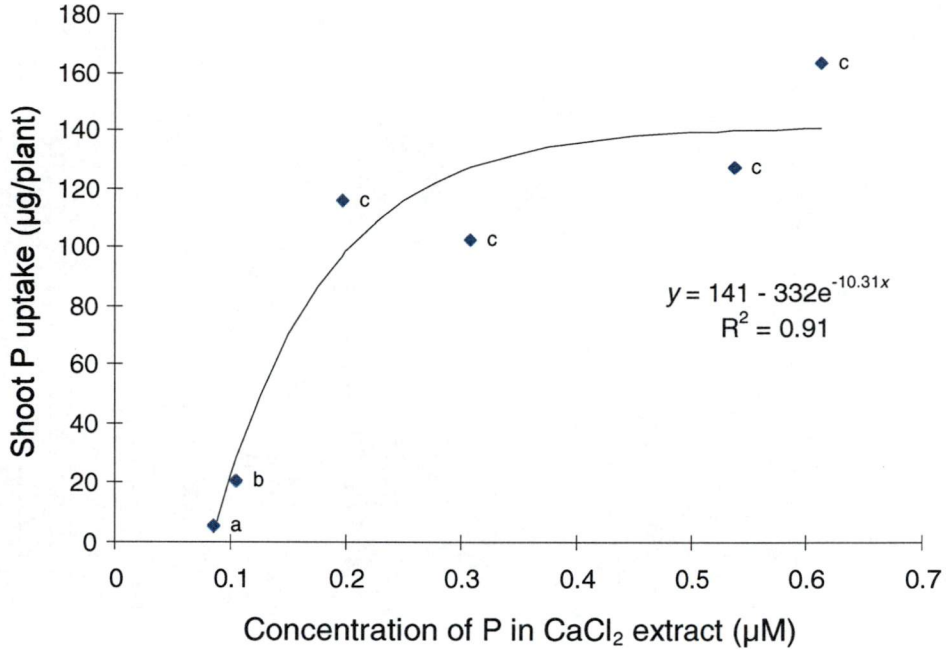
A Mitscherlich fit of tissue P concentration (Figure 6.10, $y = 0.00472\text{-}0.012e^{-12.86x}$) reached an asymptotic growth level at a higher $CaCl_2$ P than tissue N concentration, and indicators of growth response, with 90% of maximum predicted tissue P concentration occurring at a $CaCl_2$ P concentration of 0.25 µM.

Figure 6.10 - Shoot P concentration with increasing $CaCl_2$ P. Treatments with the same letter were not significantly different.



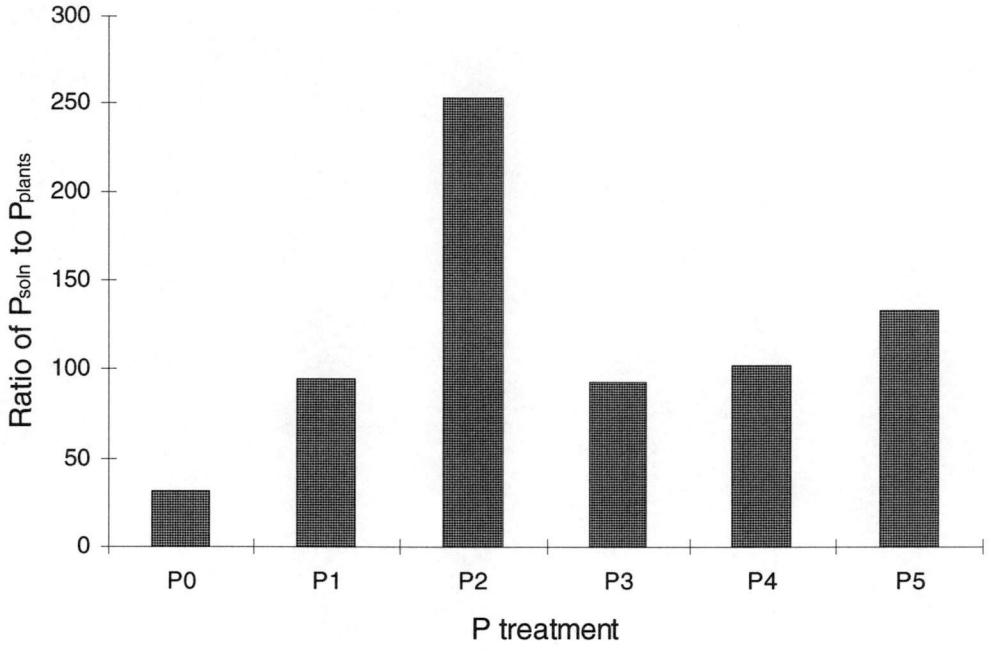
A Mitscherlich function gave a poorer fit of the relationship between $CaCl_2$ P and P uptake per plant ($R^2 = 0.91$), because a plateau in P uptake was less apparent than for the other growth traits (Figure 6.11). Variability was too high for analysis of variance to show any significant difference between the four highest treatments, but there was a significant difference in P uptake between the first three treatments.

Figure 6.11 - Relationship between P uptake and $CaCl_2$ P concentration.



Uptake by plants during the experiment was compared with the quantity of P in the soil solution pool at any one time, to estimate the relative contribution of the quantity and intensity pools of soil P. Plants in each of the P fertilized treatments acquired more than 90-fold the quantity of P instantaneously available in the solution pool (Figure 6.12). There were no significant differences between treatments.

Figure 6.12 - Ratio of P taken up by plants (P_{plants}, µmol/pot) to instantaneous P in soil solution (P_{soln}, µmol/pot) at each P treatment level.



The P_2 treatment (in which the optimum solution concentration of 0.20 µM was attained) had the highest relative extraction ratio of P from the soil (~250). The ratio stayed at approximately 100 for the other fertilized treatments.

Specific absorption rates for *E. nitens* roots in this experiment ranged from 1.73×10^{-6} /day in the P_0 treatment to 1.06×10^{-5} /day in the P_5 treatment (Table 6.11).

Table 6.11 - Specific absorption ratios of P in each treatment.

Treatment:	P_0	P_1	P_2	P_3	P_4	P_5
Estimated SAR[A] (/day):	1.73×10^{-6}	3.46×10^{-6}	7.49×10^{-6}	8.44×10^{-6}	9.24×10^{-6}	1.06×10^{-5}

The volume of soil in depletion zones around plant roots occupied between 0.05 and 0.33% of total soil volume in each pot (Table 6.12).

Table 6.12 - Percentage volume of pots occupied by P depletion zone around *E. nitens* roots.

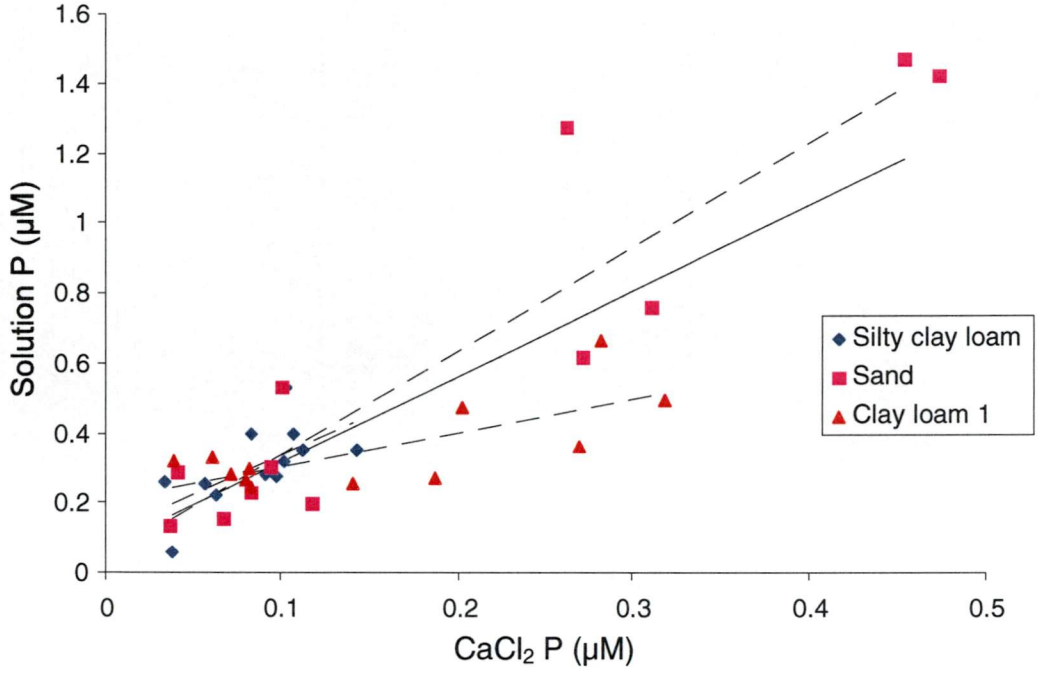
Treatment	P_0	P_1	P_2	P_3	P_4	P_5
Depletion zone (% of soil volume)	0.06	0.11	0.25	0.28	0.30	0.33

6.3.2 Pot experiment 2: Growth and P uptake of *E. nitens* in soils of different P-sorption characteristics.

The relationship between $CaCl_2$ P and solution P was significantly correlated within each soil type. Equations for the regressions were: $y = 2.97x + 0.041$ ($R^2 = 0.85$, $P < 0.01$) for the sand, $y = 0.95x + 0.21$ ($R^2 = 0.53$, $P < 0.05$) for the clay loam 1, $y = 2.28x + 0.11$, ($R^2 = 0.41$, $P < 0.05$) for the silty clay loam. The regressions were not significantly different from each other, and the relationship for all soils was described by a common linear regression:

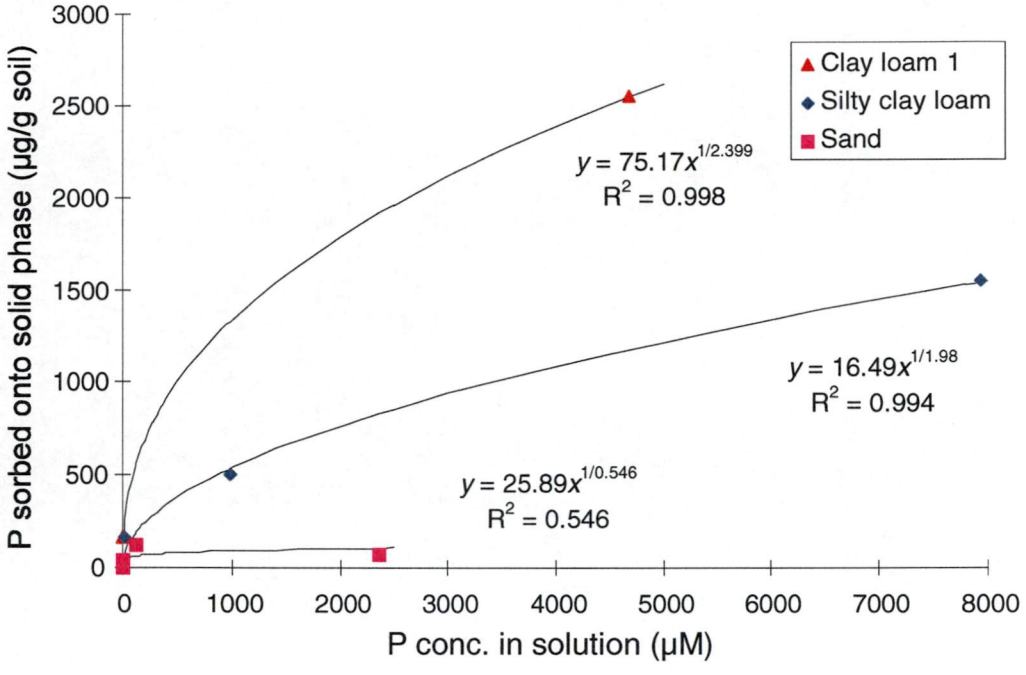
$y = 2.44x + 0.075$ ($R^2 = 0.72$, $P < 0.01$)

Figure 6.13 - Relationship between solution P and $CaCl_2$ P for three soils of different P sorption characteristics. Dashed lines show regressions for individual soils, and the solid line shows overall regression.



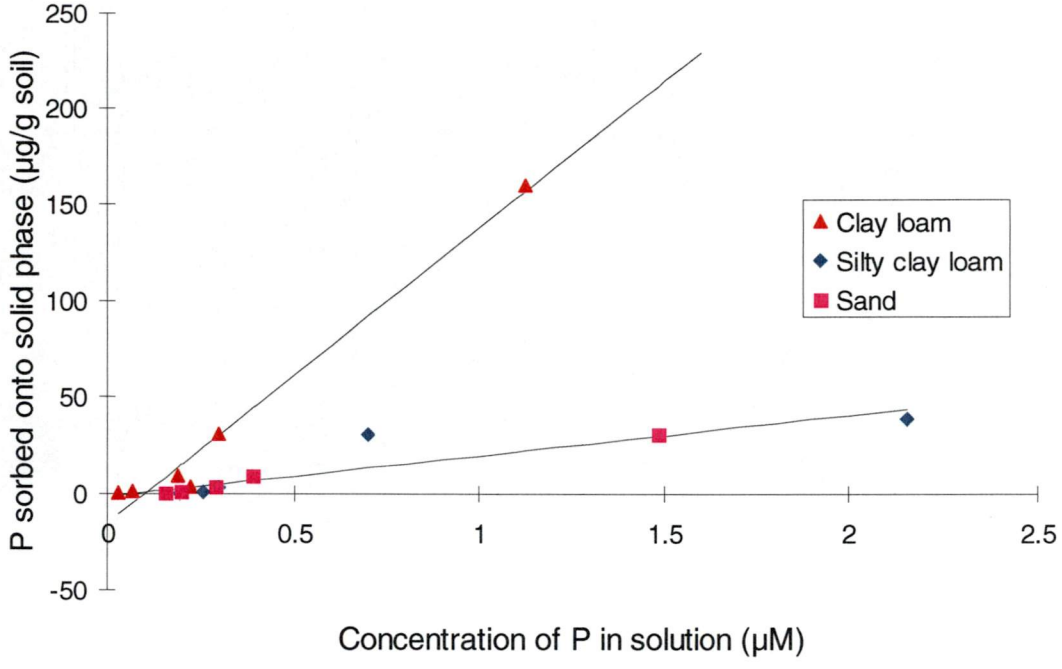
Sorption curves were used to describe the P buffering characteristics of soils in this experiment (Figure 6.14). A Freundlich function fitted the data well for the silty clay loam and clay loam 1 ($R^2 > 0.99$), but the P sorption curve for the sand was more variable ($R^2 = 0.55$), because error caused by dilution (± 50 µg/g) at higher concentrations of P in solution was similar to the amount of P sorbed (~100 µg/g).

Figure 6.14 - Sorption curve for the three soils over the full range investigated.



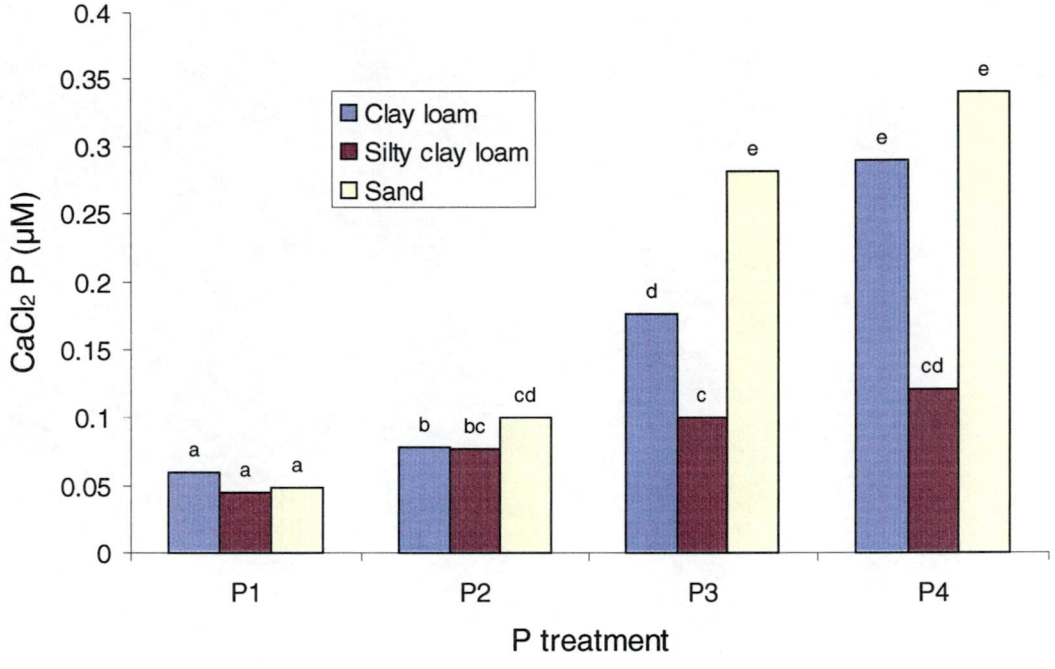
While full sorption curves were useful for indicating overall sorption characteristics of the soils, the concentration range of interest for plant growth was between 0 and 2 µM P in solution. The sand and silty clay loam soils sorbed a similar quantity of P over the 0-2 µM P range (Figure 6.15), and P sorption was described by a common linear regression ($y = 20.94x - 1.056$), which explained 83.2% of the variance. A separate linear regression ($y = 152.63x - 14.921$) explained 97.8% of the variance in sorption of the clay loam 1 soil over the 0 to 2 µM solution P range.

Figure 6.15 - Sorption curve of the three soils over the concentration range of interest (up to 2.5 µM)



Concentrations of $CaCl_2$ P achieved in the experiment ranged from 0.05 µM to 0.3 µM (Figure 6.16), and covered the hypothesised optimum of 0.2 µM in two of the soils. Analysis of variance of the log-transformed data showed highly significant soil type ($P < 0.001$) and treatment ($P < 0.001$) effects. The clay-loam 1 was the only soil with a significantly greater concentration at each treatment level. The sand soil had an increasing trend with each P treatment, but no significant difference was measured between concentrations at the highest two treatments. There was no significant difference between the final three treatments for the silty clay loam, although an increasing trend with each P treatment was evident.

Figure 6.16 - Concentrations of $CaCl_2$ P obtained. Columns with the same letter were not significantly different (P = 0.05).



A decreasing trend with $CaCl_2$ P over time was observed in all treatments with added fertilizer, especially the sand and silty clay loam (Figure 6.17). The magnitude of the actual decrease was probably no more than 0.1 µM P during the experiment.

Colwell extractable P showed similar downward trends over time in all soils (Figure 6.18), but the only significant regression occurred with the P_3 treatment in the sand soil ($y = -0.225x + 39.87$, $R^2 = 0.94$, $P < 0.05$).

Figure 6.17 - Change in $CaCl_2$ P with time in the silty clay loam (a), sand (b), and clay loam 1 (c). The bar shows least significant difference (P = 0.05) between treatments at final harvest.

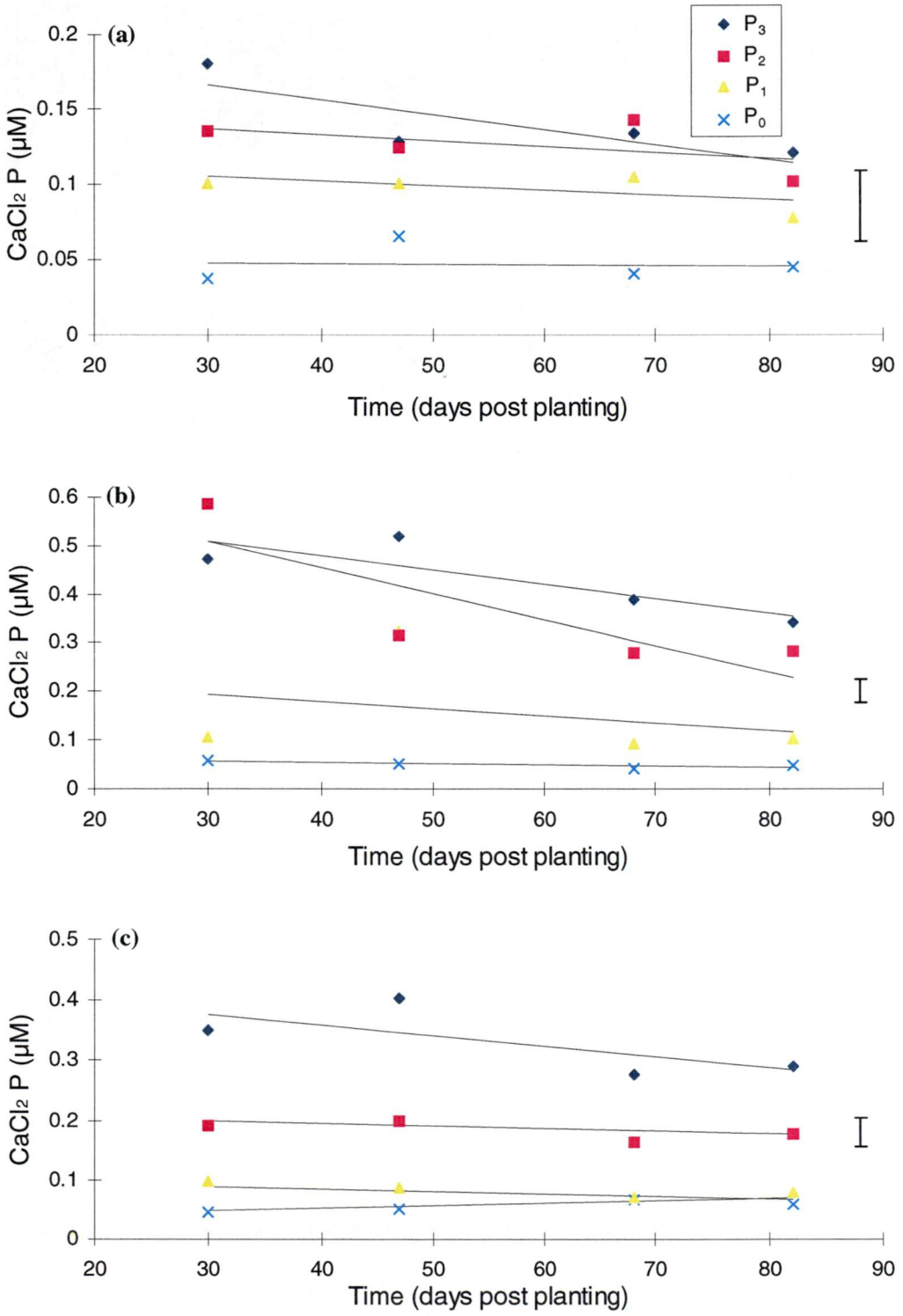
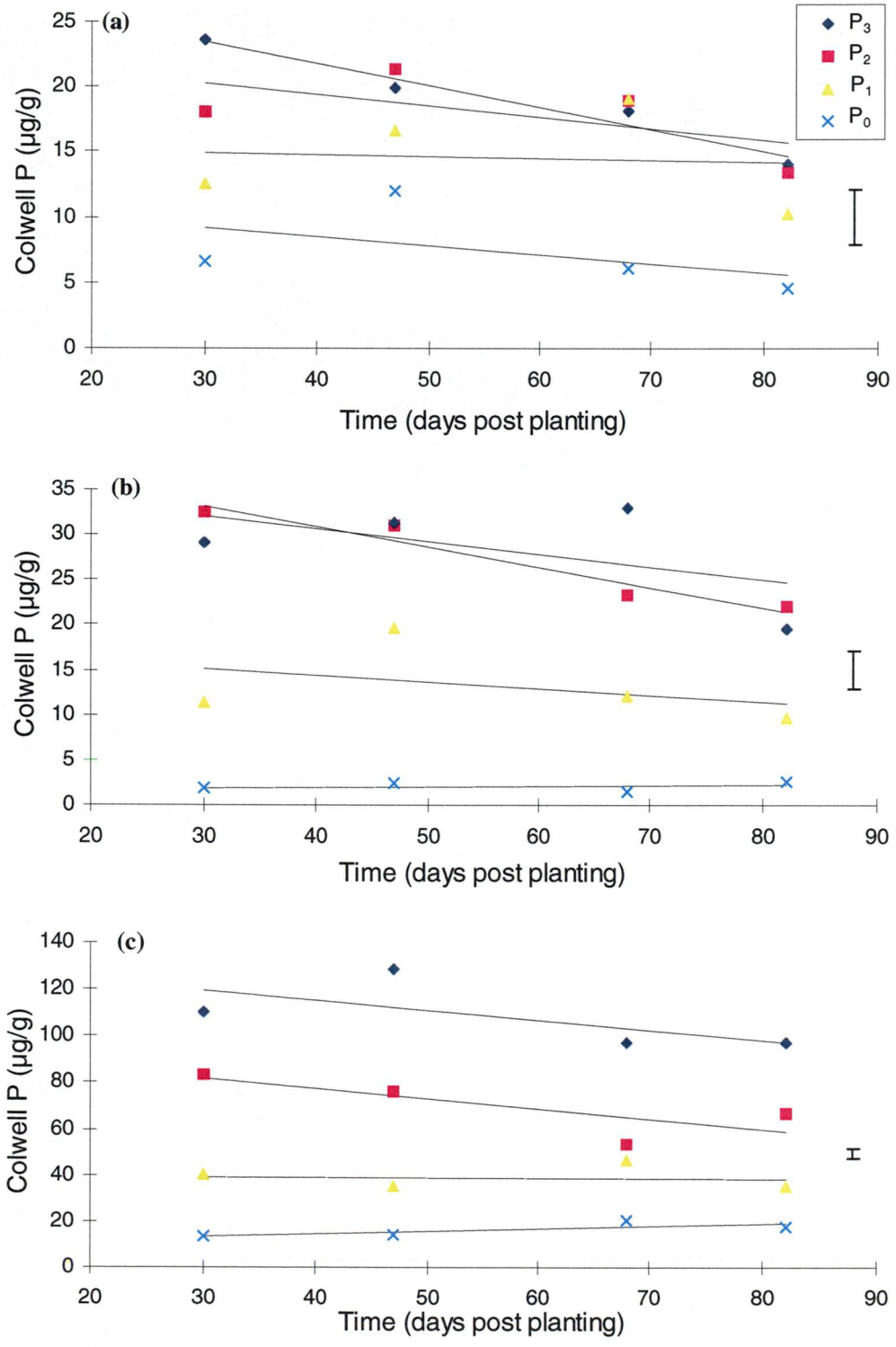
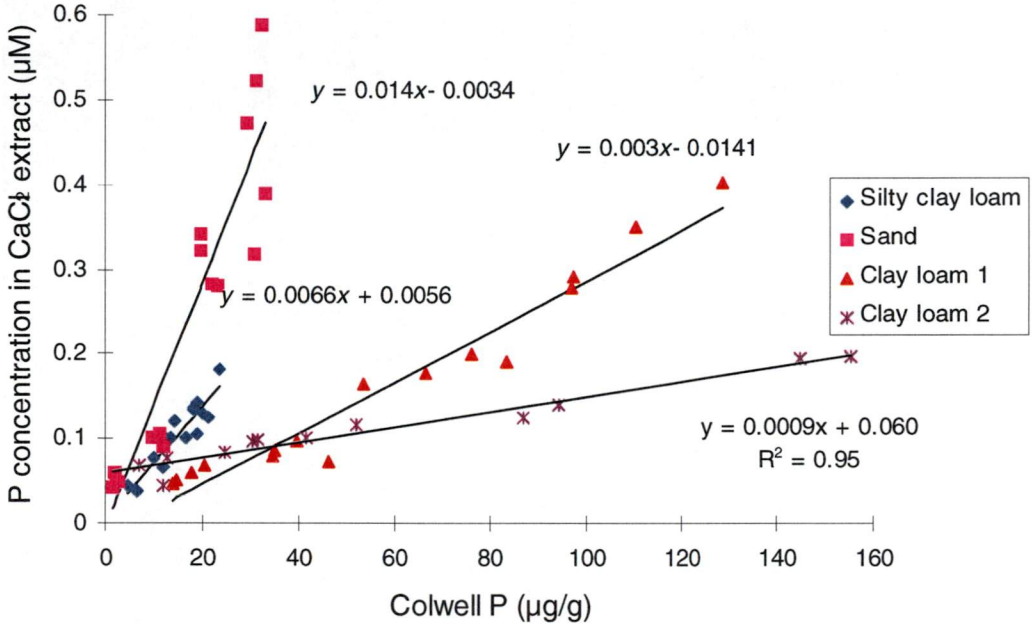


Figure 6.18 - Colwell P over time in the silty clay loam (a), sand (b), and clay loam 1 (c). The bar shows least significant difference (P = 0.05) between treatments at final harvest.



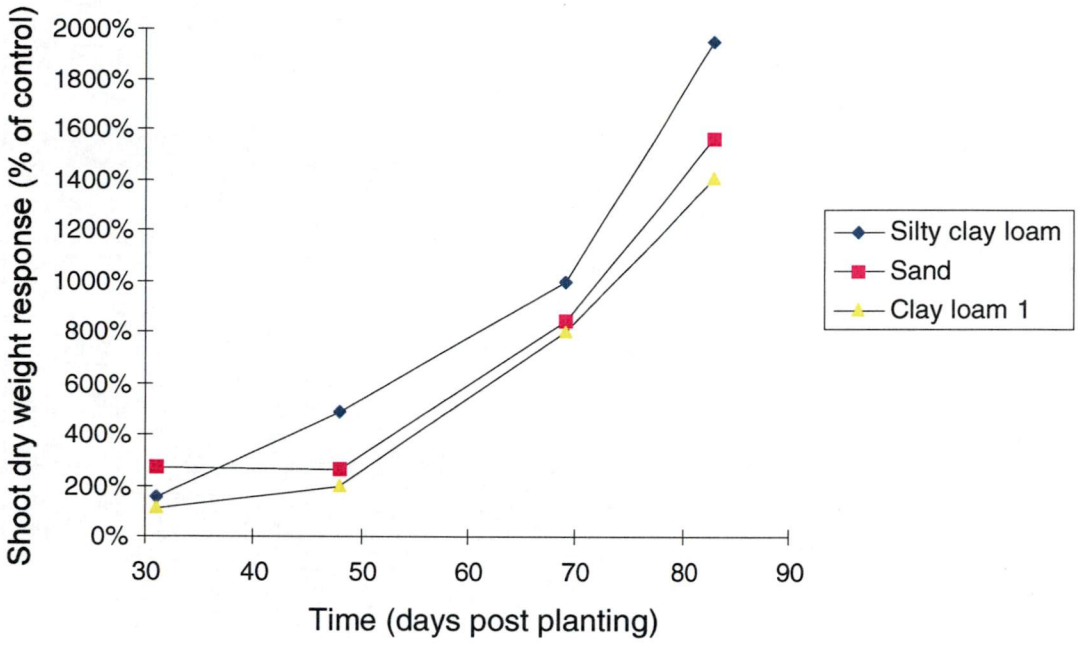
The relationship between Colwell P and $CaCl_2$ P was close to linear for each soil type, but different between soil types (Figure 6.19). The slopes of the regression lines were 0.0009 µM per µg/g Colwell P for the clay loam 2, 0.003 µM per µg/g Colwell P ($R^2 = 0.95$) for the clay loam 1, 0.0066 µM per µg/g Colwell P ($R^2 = 0.86$) for the silty clay loam, and 0.014 µM per µg/g Colwell P ($R^2 = 0.86$) for the sand. Increasing slope approximately correlated with decreasing buffer power (see Table 6.4).

Figure 6.19 - Relationships between Solution P and Colwell P for the 4 soils.



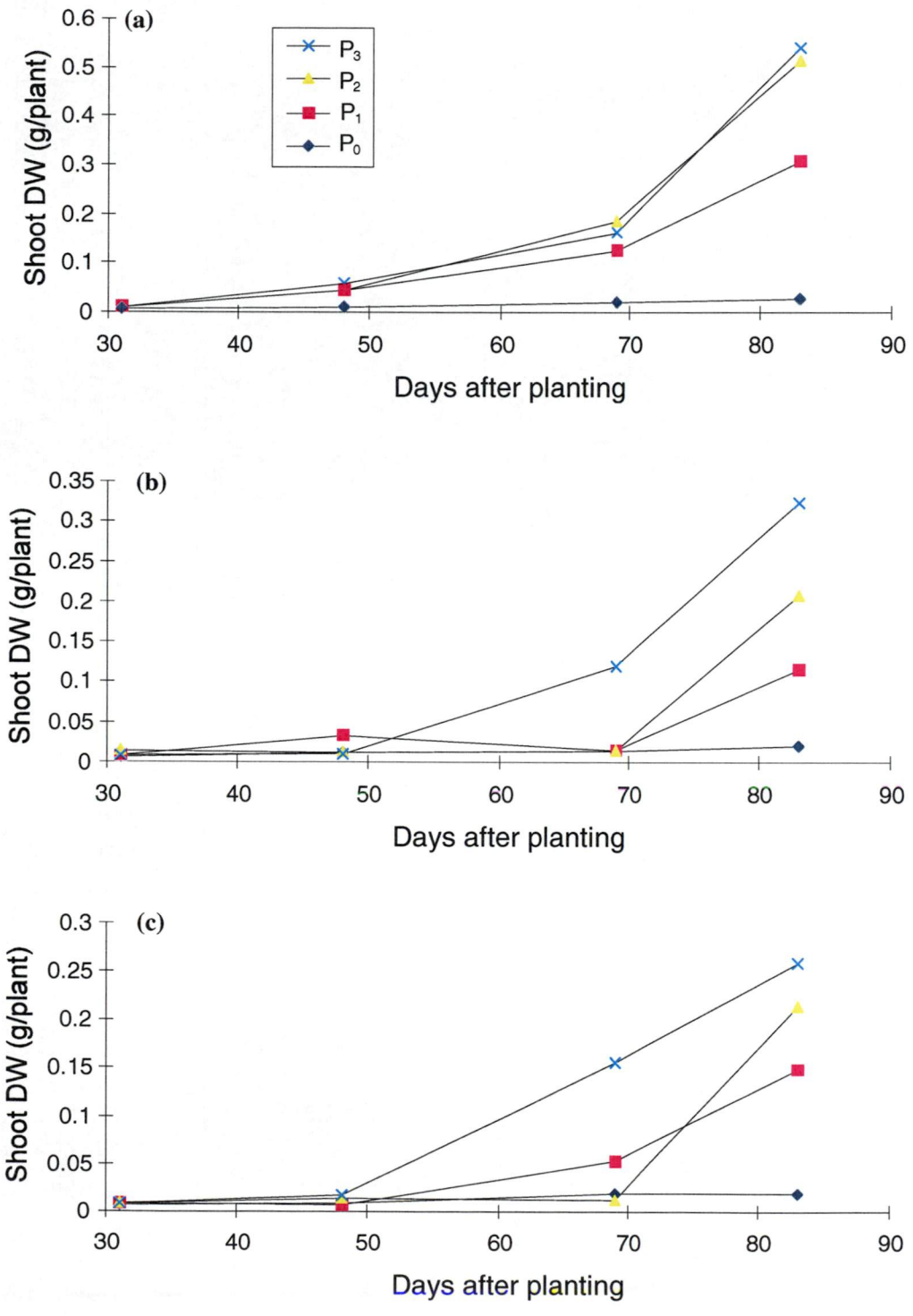
Shoot dry weight response (ie. percentage increase over control, Section 6.2.2.3) increased over time (Figure 6.20), and was similar for each soil type.

Figure 6.20 - Shoot dry weight response in each soil type.



Dry matter accumulation over time in each soil type is shown in Figure 6.21. Dry weight in the control treatments remained close to constant during the experiment, while plants with optimum P levels showed an exponential growth increase over the time of the experiment. Lack of differentiation between treatments in the silty clay loam gave similar response curves for each treatment (Figure 6.21a), but response curves for the other two soils were different at each treatment level (Figure 6.21b and c).

Figure 6.21 - Shoot dry weight accumulation in the silty clay loam (a), sand (b), and clay loam 1 (c) soils.



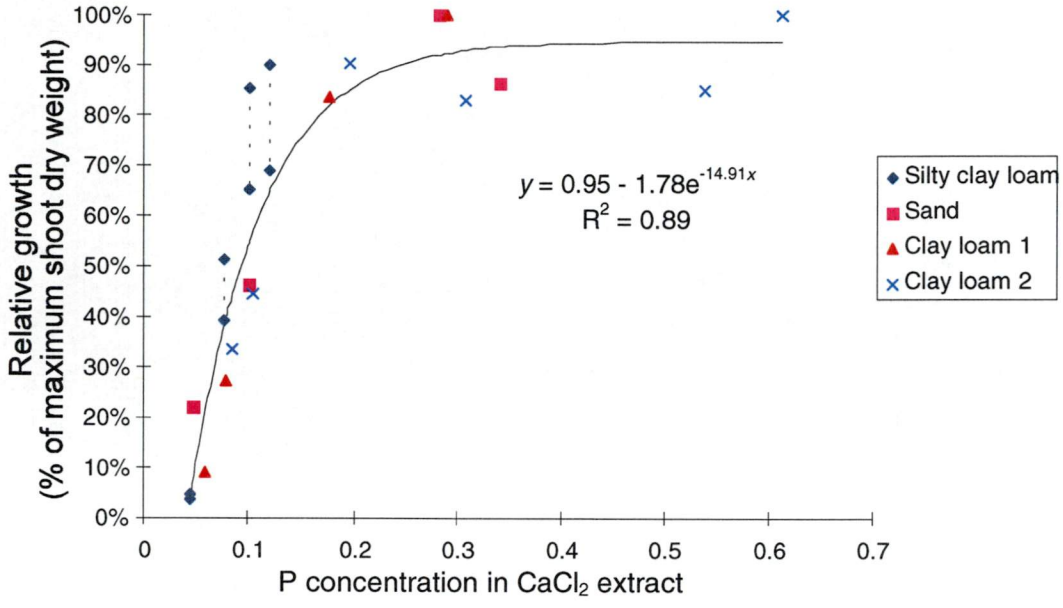
Treatments with the most fertilizer in the sand and clay loam 1 soils responded earliest.

Shoots in the unfertilized treatment remained at, or near to, the same weight that they were at transplanting.

To compare response to P between soil types, relative growth was calculated as a percentage of the predicted maximum. Relative yield was also calculated for the first pot experiment, with the clay loam 2 soil. Plant dry weights in the sand and clay loam 1 soils reached an asymptotic level with treatment, but there was no apparent asymptote for growth in the silty clay loam (where maximum $CaCl_2$ P reached only ~0.12 μM), although dry weight in the top two treatments was not significantly different. Hence, an assumption was made about the probable range of maximum shoot dry weight in that soil, in order to compare relative growth in that soil with the other soils. It was assumed that the actual maximum dry weight that would have been achieved in the silty clay loam soil was between 0.6 and 0.8 g/plant.

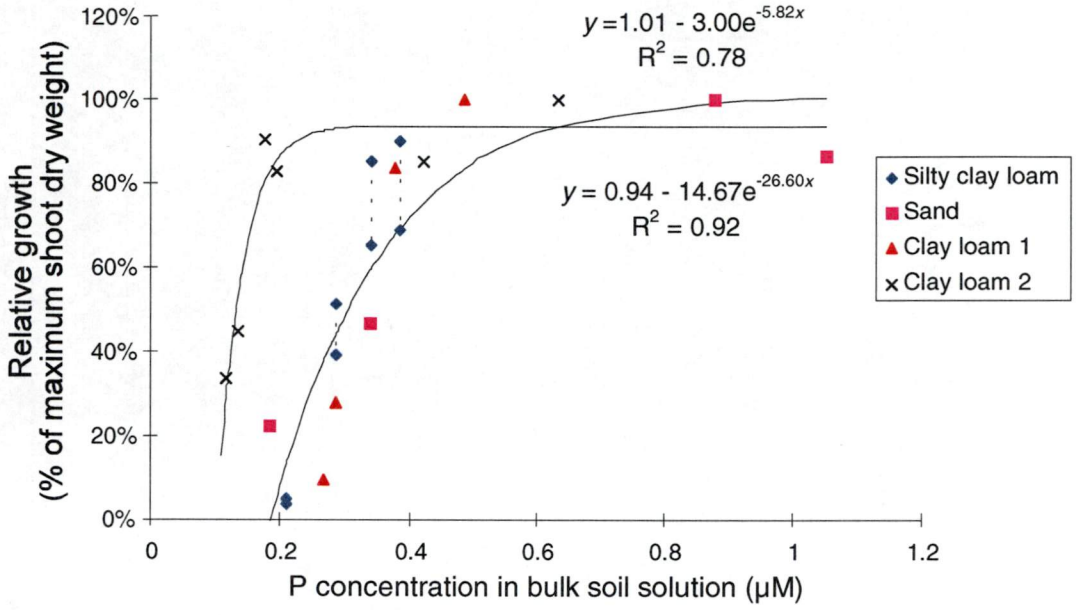
A single Mitscherlich model ($y = 0.94 - 1.94e^{-16\,88.x}$) accounted for 89% of the variance in the relationship between $CaCl_2$ P and relative growth (Figure 6.22) in all 4 soils (including growth in the clay loam 2 from the first pot experiment). The value of $CaCl_2$ P at 90% of maximum relative growth response was 0.21 μM.

Figure 6.22 - Relationship between relative growth response and $CaCl_2$ P for the soils from pot experiments 1 (clay loam 2) and 2 (silty clay loam, sand and clay loam 1). The probable range of the silty clay loam is shown with dashed-lines; the average of the range was used in the regression.



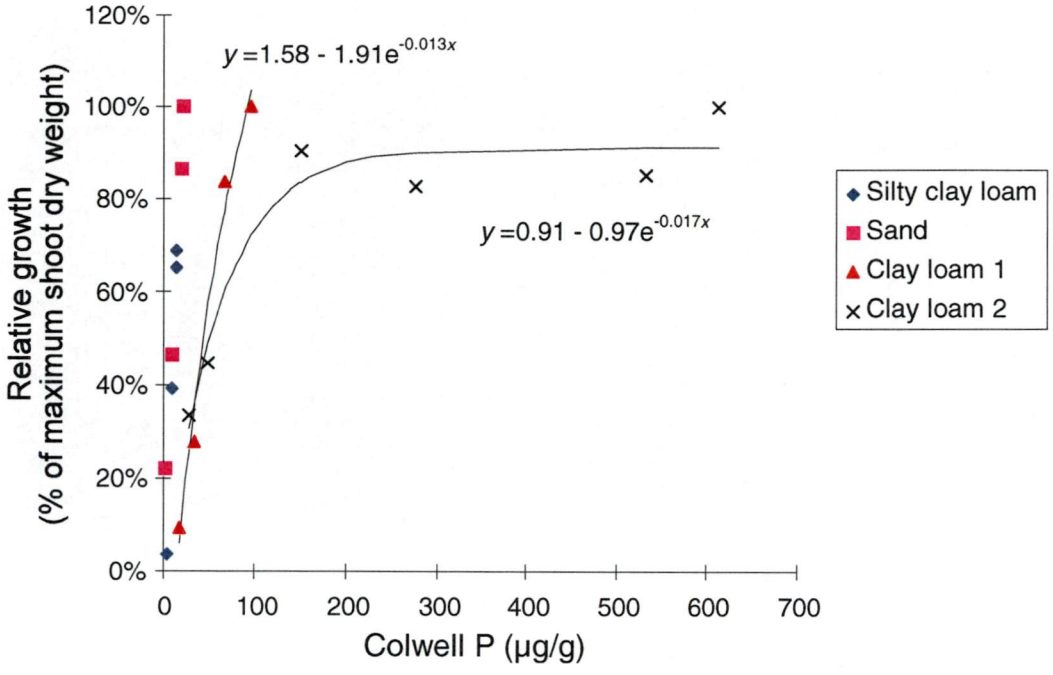
The relationship between growth response and concentration of P in bulk soil solution was not as highly correlated as with $CaCl_2$ P (Figure 6.23). A common regression for all soils (not shown) described 38.2% of the variance ($P < 0.01$), but the clay loam 2 soil (from the first pot experiment) had a lower optimum concentration than the other soils. A Mitscherlich fit of the soils from the second experiment described 78.1% of the variance ($P < 0.01$). A second regression was derived to fit relative growth response in the clay loam from the first pot experiment (clay loam 2), which described 92% of the variance. Critical solution P concentrations were 0.19 µM for the clay loam 2 soil, and 0.582 µM for the soils from the second pot experiment.

Figure 6.23 - Relationship between relative growth response and concentration of P in soil solution. The probable range of the silty clay loam is shown with dashed-lines; the average of the range was used in the regression.



Relative growth response was highly correlated with Colwell P in each soil type, but the response curve for each soil was independent of the others (Figure 6.24). Asymptotes were not as evident in the Colwell P data (Figure 6.24) as they were for $CaCl_2$ P and solution P (Figure 6.22 and Figure 6.23). Mitscherlich curves were fitted for the clay loam 1 and clay loam 2 soils.

Figure 6.24 - Relationship between relative growth response (defined as percentage of maximum growth) and Colwell P for 4 soils from this experiment.



Specific absorption ratio increased with P treatment in each soil type, and ranged between 1.31×10^{-5} and 1.24×10^{-3} /day (Table 6.13).

Table 6.13 - Specific absorption ratios of P in each treatment of pot experiment 2.

Soil	P_0	P_1	P_2	P_3
Silty clay loam	2.72×10^{-5}	1.24×10^{-4}	2.33×10^{-4}	2.63×10^{-4}
Clay loam 1	2.08×10^{-5}	3.47×10^{-4}	4.07×10^{-4}	8.24×10^{-4}
Sand	1.31×10^{-5}	1.80×10^{-4}	3.32×10^{-4}	1.24×10^{-3}

Depletion zone volumes around roots in each pot ranged between 0.36 and 78% of the pot (Table 6.14), indicating that competition for P between roots would have been minimal.

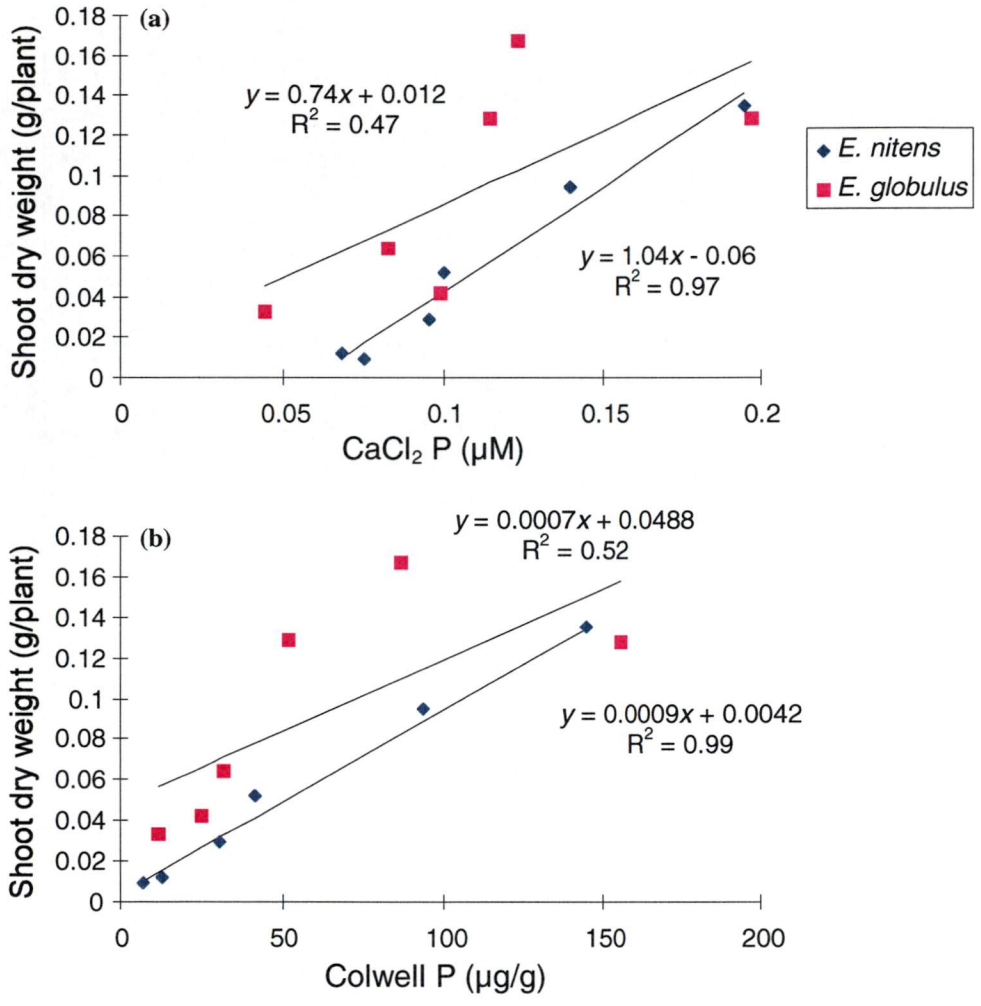
Table 6.14 - Volume of soil in the depletion zone around roots in each soil type (percent of pot volume).

Soil	P_0	P_1	P_2	P_3
Silty clay loam	13.60	49.70	61.88	77.67
Clay loam	4.43	15.84	37.23	38.66
Sand	0.36	1.38	5.64	6.65

6.3.3 Pot experiment 3: Comparison between *E. nitens* and *E. globulus* growth responses to soil P.

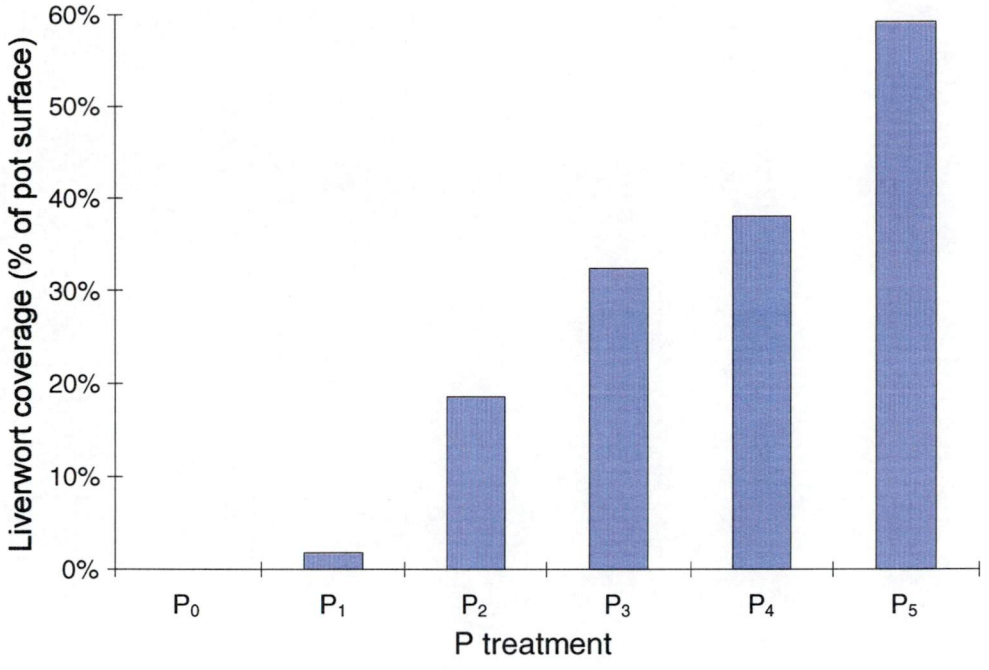
Regressions between solution P and shoot dry weight for both species were not significantly different (Figure 6.25a), but the $CaCl_2$ P concentrations obtained were lower than planned, and did not exceed the hypothesised 0.2 μM critical concentration. Variability in plant dry weight was high, because (a) poor survival of *E. globulus* seedlings in the germination phase left only enough for 1 seedling per pot, and (b) P concentrations were approaching the sensitivity limit (0.05 μM) of the methods employed, so variability in measured P concentration was high. Dry weights of *E. globulus* shoots were generally higher than those of *E. nitens*, because *E. globulus* seeds initially weighed more than *E. nitens* seeds (2 mg versus 0.5 mg/seed). The relationship between Colwell P and shoot dry weights was also very similar for the two species (Figure 6.25b), with no significant difference between the slopes of the two regression lines.

Figure 6.25 - Relationship between shoot dry weight of *E. nitens* and *E. globulus* and $CaCl_2$ P (a) and Colwell P (b).



Liverwort coverage in each pot increased with P treatment (Figure 6.26), indicating that P was limiting growth of liverworts, as well as *Eucalyptus* seedlings.

Figure 6.26 - Liverwort coverage of pot surface area in each treatment.



6.4 Discussion

Moody *et al.* (1988) found a linear relationship between solution P concentration and $CaCl_2$ extractable P ($R^2 = 0.89$) for a wide range of soils and P concentrations (0.13 - 133 µM P in soil solution). The slope of the regression was calculated to be 0.67 µM $CaCl_2$ P/µM solution P, indicating that $CaCl_2$ P consistently underestimated P concentration in solution. A significant overall linear relationship was found for the silty clay loam, clay loam and sand soils in the current experiment (Figure 6.13, $R^2 = 0.72$; range of concentration was 0.05 - 1.5 µM P in solution), but within each soil type, the slope of the regression appeared to be partly influenced by soil P buffer power. Concentration of P in the $CaCl_2$ extract was less in soils with lower buffer powers.

A curvilinear relationship was found between $CaCl_2$ P and solution P in the highly P-sorbing clay loam (Figure 6.1). The concentration of P in the paste extract and the $CaCl_2$ extract were similar below 0.2 µM, and at 0.6 µM, but between 0.2 and 0.6 µM P, paste P and $CaCl_2$ P

appeared to measure different pools of P. The mechanism for this was unknown, but was probably caused by differences between the paste and $CaCl_2$ extract methodologies. The $CaCl_2$ extract involved soil drying, longer extraction period, and presence of Ca^{2+} ions during extraction. Barrow and Shaw (1979) showed that increased time of extraction resulted in increased desorption (and hence greater concentration in extracting solution), while increased Ca^{2+} concentration resulted in decreased desorption. The high solution:soil ratio of the $CaCl_2$ extract (10:1) may have caused low concentration of P in soils with lower buffer power, but Smethurst *et al.* (1997) found little effect of dilution (up to 16-fold) on the concentration of P during development of the paste extract for the silty clay loam, clay loam 1 and clay loam 2 soils used in this experiment. The $CaCl_2$ extract was more applicable to routine laboratory analysis because it allowed more samples to be processed per day than the paste extract (60 /day *cf.* 24 /day), used less soil per sample (5 g *cf.* 240 g), and the soil could be air dried and stored before extraction (fresh soil was required for the paste extract). Solution P concentration in subsequent analyses was inferred from the $CaCl_2$ P concentration.

Concentrations of $CaCl_2$ P in all pot experiments were lower than planned (Figure 6.4, Figure 6.16), probably due to slow sorption by soil, which was not accounted for by the 17 hour sorption analysis. Slow sorption is a common phenomenon in soils, which is poorly accounted for by short-term sorption curves (eg. Barrow 1989). Even though concentrations achieved were lower than planned, the range of $CaCl_2$ P covered the critical concentration for growth of *E. nitens* in 3 of the 4 soils (Figure 6.22). A highly correlated common relationship between growth response and $CaCl_2$ extractable P was observed for all four soil types (Figure 6.22). The relationship between solution P and growth response was not as distinct (Figure 6.23), but had significantly less soil-type specificity than the relationship between growth response and Colwell P (Figure 6.24). Holford (1991, 1997) suggested that a combination of P buffer power and intensity was required for a P analysis to be successful, and it appeared that the $CaCl_2$ extract gave the right combination of P buffer power and intensity for prediction of P deficiency in eucalypts.

Shoot weight and leaf area were most sensitive to P deficiency, so optimum $CaCl_2$ P for *E. nitens* seedlings was best indicated by those two factors. The optimum $CaCl_2$ P of 0.21 ± 0.05 µM was equivalent to 0.18, 0.59, 0.66, and 0.41 ± 0.05 µM P in soil solution for the clay loam 2, silty clay loam, sand and clay loam 1 soils, respectively. These critical concentrations were at the lower end of the published range of critical external concentrations of P required for optimal growth (Table 2.4). The low P requirement was not due to poor plant growth, as relative growth rates (between 0.048 and 0.065, Table 6.9) were consistent with values published by Barrow (1977) for a number of native Australian plants. The relative growth rates that Barrow (1977) observed ranged from 0.026 for P sufficient *Banksia grandis*, to 0.055 for P sufficient *Eucalyptus diversicolor*. Kirschbaum *et al.* (1992) found a maximum relative growth rate for solution cultured *E. grandis* of 0.08 /day.

Species with a similar published critical soil solution P concentrations were cassava (*Manihot esculenta*) in a field experiment (Fox 1981), and soybeans in a field experiment (Moody *et al.* 1983). The low solution P requirement of cassava in that experiment may have been related to high dependence by cassava on association with vesicular-arbuscular mycorrhizae (Howeler 1990), because a concentration of 28-78 µM was required for optimal growth in (non-mycorrhizal) flowing solution culture (Jintakanon *et al.* 1982). Published solution P requirements of most species were between 1 and 10 µM (Table 2.4), so *E. nitens* roots had a comparatively low requirement for external P concentration. The hypothesis of Handreck (1997) that Australian native species have evolved to efficiently utilize P from the low P soils of Australia was supported by the low concentration requirement of *E. nitens* for P. Liverwort coverage of the pots was also influenced by P treatment (Figure 6.26), indicating that P concentrations were also below optimum for Liverwort growth.

The optimum concentration of P in soil solution for *Pinus radiata* seedlings was approximately 6 µM (Skinner and Attiwill 1981), significantly higher than the 0.2-0.6 µM found for *E. nitens* in this experiment. The optimum concentration found by Skinner and

Attiwill (1981) may have been over-estimated, because they did not measure the concentration achieved during the experiment, but rather predicted it from a sorption isotherm. If the P concentration reduction in the Skinner and Attiwill (1981) experiment was similar to that observed in the current experiment (ie. to about 35% of desired for the Ferrosol, see Figure 6.4), the optimum solution concentration for *P. radiata* would have been approximately 2 μM, 10-fold above the 0.2 μM critical concentration for *E. nitens* found in this experiment. The requirement for higher P concentration in soil solution may explain why *Pinus radiata* has poorer growth at sites where eucalypts can grow vigorously (Huang *et al.* 1991).

The ratio of P taken up to the quantity in solution at any one time (Figure 6.12) showed that the solution pool of P was replenished many times during the course of the experiment. The ratios in Figure 6.12 were conservative estimates, because they accounted for all soil in the pot, rather than only soil in depletion zones around roots. However, soil volume in the depletion zones of roots in fertilized soil would have been underestimated, because buffer power in those treatments would have been overestimated by the adsorption curve (see Section 2.2.2.2). Soil volume in the calculated depletion zone around roots in the clay loam 2 of the first pot experiment was only 0.05 - 0.33% of the soil volume (Table 6.12), so the ratio of quantity taken up to quantity in the soil volume explored by roots was >300 fold more than shown in Figure 6.12. It can be inferred that the seedlings were dependent on release of P from the solid phase, but despite this, growth was well correlated with the intensity component of soil P. Similar ratios of P taken up to the instantaneous quantity of P in the solution pool was calculated for other published experiments. *Triticum aestivum* extracted between 40 and 678 times that available in the soil solution pool after 21 days of growth in a pot experiment (Gardiner & Christensen 1990), and *Pinus radiata* extracted between 62 and 315 times that available in solution at any one time after 7 months of growth (Skinner and Attiwill 1981). Holford and Mattingly (1976) found that soil P intensity was correlated better with early than later growth of ryegrass (R^2 = 0.53, 0.35 and 0.23 at 40, 77 and 117 days after

sowing, respectively). The approximate ratio of P taken up to P in solution in that experiment was calculated to be between 300 and 1300 at day 40, and increased to between 900 and 5500 at 117 days after sowing. Hence the *E. nitens* seedling roots in this experiment may not have exploited the soil enough for the quantity component of soil P to become the limiting factor for growth.

If P supply to roots was limiting P uptake, then a possible plant strategy to increase P uptake could be to extend roots into undepleted soil volumes. The undepleted soil volume in the first experiment was greater than 99% at 64 days (Table 6.12), while in the second pot experiment, the volume of soil in undepleted volumes of soil ranged between 23 and 99% at 86 days (Table 6.14). The reason for the much greater exploration of soil by roots in those treatments was the greater depletion zone widths, generally caused by lower buffer powers of the soils involved. The volume of soil in the depletion zone of the sand was lowest, despite the low buffer capacity (Table 6.4), because of the low water content of that soil ($\theta_v = 0.05$, Table 6.6; Equations 6.1, 6.2 and 6.3). Undepleted volumes of P were available in all soils, and extension of roots into those areas would aid plant acquisition of P. In the field, a greater soil volume is available to roots, so a considerable time may elapse before roots have no more undepleted zones to occupy, especially in soils of high P buffering ability.

The low diameter of *E. nitens* roots in the current study was similar to other observations on *E. nitens* roots (Misra and Gibbons 1996), indicating that they were adapted for allocation of carbon to length, possibly for increased soil exploration. The observed root diameters (< 0.2 mm) were equivalent to the smallest (ie. for *E. diversicolor*) observed by Barrow (1977), and Fitter (1985) noted that root diameter generally decreased with nutrient deprivation. Mycorrhizae also increase effective root length at relatively low carbon cost. Another potential advantage of increased soil exploration is the possibility of finding microsites of increased P availability. This was hypothesised to occur in a non-reported preliminary experiment with *E. nitens* seedlings in the highly P-buffered clay loam 2 soil. After several

months, most of the seedlings in the unfertilized treatments had minimal shoot growth, but two seedlings were large and healthy. The root system of the healthy plants was extensive, with several root clusters. It was postulated that the root clusters were utilizing microsites of high P availability, probably in the form of decomposing organic matter. Genetic differences in uptake ability between the seedlings would also explain the difference in P nutrition. For example, the seedlings that grew well may have had a low K_m. Genetic variation was hypothesised to be the reason for the high variation in P-32 uptake studies (Chapter 4).

The specific absorption rate of P by *E. nitens* roots in this experiment was similar to SAR's of several Australian native seedlings, indicating that the *E. nitens* roots had a similar effectiveness in acquiring phosphate. Barrow (1977) observed SAR's between 0.0003 and 0.0015 /day, while the SAR's for *E. nitens* in this experiment were between 1.73×10^{-6} and 0.00124 /day (Table 6.11, Table 6.13). Specific absorption ratios varied with soil type, increasing in the following order: clay loam (high P sorbing) < silty clay loam < clay loam (medium P sorbing) < sand. The SAR was generally inversely proportional to buffer capacity, with the silty clay loam an exception. The silty clay loam was poorly structured, and roots were generally restricted to existing cracks in the soil, causing a lower than expected SAR.

The similarity of response to $CaCl_2$ P in all soil types indicated that $CaCl_2$ P may be a useful indicator of P deficiency in eucalypt plantations, applicable in soils of widely differing P buffer powers. However, the relationship between growth response and P intensity is only highly correlated soon after planting in ryegrass (Holford and Mattingly 1976), and soil P quantity indicators were better correlated at increasing times after planting (eg. after 40 d) because the capacity of the soil to supply P became the growth limiting factor. The close relationship between growth response to P and soil P intensity in the current experiment may have been because of the short duration of the experiment, or it may be that eucalypts respond to P intensity irrespective of the duration of growth, as has been found for soybeans (Moody *et al.* 1983), subterranean clover (Dear *et al.* 1992), and a range of other crops (Fox

1981). The main response to P fertilizer in *E. nitens* occurs during the first year after planting (Chapter 2), which is the time-scale of the agricultural crops mentioned above. The relationship between P indicators and eucalypt response to P fertilizer over longer time-scales (1 year) was investigated using field experiments in the next chapter (Chapter 7).

Colwell P values were related to soil buffer power (ie. high buffer power gave high Colwell P result, Figure 6.19). The Colwell, or other quantity-based indicators would be useful for predicting response within a soil type, but empirical experiments would be needed to calibrate test values with response in each soil type. All common indicators of crop P deficiency need to be empirically tested in such a way (Holford 1997). The Colwell test may prove to be more useful than direct intensity measures for predicting response to P over longer time-scales, because it incorporates more of the quantity component of soil P availability (Holford 1997). The slope of the relationship between $CaCl_2$ P and Colwell P appeared to be inversely related to buffer capacity (Table 6.4, Figure 6.19), so incorporation of both Colwell and $CaCl_2$ P data, or Colwell and buffer capacity data may improve prediction of P deficiency over the longer term, as found for ryegrass (Holford and Mattingly 1976) and wheat (Holford and Cullis 1985).

Eucalyptus globulus and *E. nitens* seedlings responded similarly to soil P levels in this experiment, but evidence was equivocal, due to high variation and low range of $CaCl_2$ P. Supporting the assumption that *E. nitens* and *E. globulus* respond similarly to P, Adams *et al.* (1995) found no significant difference in P response between the two species in a pot experiment, and Judd *et al.* (1991) found no significant differences in foliage concentrations of P. Bennett *et al.* (1996) found that *E. globulus* responded to higher levels of P fertilizer than *E. nitens*, but this was not surprising, because the *E. globulus* site in that comparison was an ex-pine plantation, while the *E. nitens* site was ex-agricultural. Nutrient availability has been shown to be low in ex-pine sites relative to ex-agricultural sites (Wang *et al.* 1996a).

In conclusion, P intensity (solution P, $CaCl_2$ P) was better correlated with growth response of

E. nitens than P quantity (Colwell P), in soils with contrasting P sorption properties. The relationship between relative growth and solution P was described by a common regression for 3 of the 4 soils, but the best analysis was $CaCl_2$ P, for which a common regression described relative growth in all soils. $CaCl_2$ P was influenced by P buffer power, and in this case it appeared to integrate P intensity and buffer capacity to a similar degree as experienced by *E. nitens* roots. There was some doubt as to the long-term predictive ability of P intensity indicators (such as $CaCl_2$ P), because P intensity has been shown to be well correlated only with early growth in some agricultural crops. The next chapters investigate the utility of various P analyses (quantity and intensity) in longer term field experiments (Chapter 7), and of the predicted concentration at the root surface (using principles of P supply and uptake) as an indicator of potential P deficiency (Chapter 8).

7. Response of *Eucalyptus nitens* and *E. globulus* to soil P: Field experiments

7.1 Introduction

Short-term experiments with *Eucalyptus nitens* (Chapter 6) suggested that indicators of both soil P quantity (eg. Colwell P), and intensity (eg. $CaCl_2$ P) could potentially be useful for predicting P deficiency in eucalypt plantations. A soil-type specific relationship between Colwell P and response to P fertilizer was found in pot experiments, but $CaCl_2$ P had less sensitivity to soil type in these experiments, and could potentially be used to test for P deficiency in a range of soil types without the need for extensive empirical calibration. It was uncertain whether growth response to P would be as well correlated with $CaCl_2$ P over longer time-scales, so relationships between soil test result and growth response in 24 P fertilizer field experiments with *E. nitens* and *E. globulus* were investigated. Sites had been established previously by several collaborators in Tasmania, Victoria, Western Australia and New South Wales, and represented a wide range of soils. A number of types of soil analyses were tested, which provided a range of extractant strengths.

The objective of this study was to compare the ability of several soil tests to identify soils on which trees would respond to P fertilizer. Rates of P fertilizer required to obtain maximal growth were not considered.

Soil sampling was conducted between 1 and 11 years after planting. It was assumed that soil analyses would be representative of those at planting, because it was unlikely that marked changes in analyses of P quantity would have occurred since planting, especially in soils with large quantities of P in the labile and non-labile phases. Supporting this assumption, little change in Colwell P or Bray No. 1 P was observed over 2 years in two unfertilized soils under permanent pasture (Friesen *et al.* 1985), and no significant change in Bray No. 2 P was observed over 3 years in a *Pinus radiata* plantation on a highly P sorbing soil (Flinn *et al.*

1982). Intensity-based indicators generally represent a small proportion of soil P, and probably don't change significantly during the first few years of plantation growth, especially in soils that have a reasonable buffer capacity. Supporting this assumption, no significant trend occurred in soil solution P concentration over 4 years in 5 unfertilized soils with a moderate to strong P buffering ability in Tasmania (P. J. Smethurst pers. comm.).

7.2 Materials and Methods

7.2.1 Sites

Sites that were used in this survey, along with species planted, establishment year, treatment structure, soil classification, number of measured trees per plot, previous land use, location (on a state basis), and collaborating institutions are shown in Table 7.1. The majority of the Tasmanian sites were established by Greg Holz of North Eucalypt Technologies, on the Ferrosols of the Surrey Hills plantation estate in north-west Tasmania. Previous land uses were native forest (*open* forest, with a grass understorey, or *mixed* eucalypt/rainforest species), *Pinus* sp. plantation (mainly *Pinus radiata*) or pasture. Stocking rates were between 1000 and 1100 stems/hectare.

The geographic locations of the experiments are shown in Figure 7.1 (mainland sites + Exton and Westfield), and Figure 7.2 (NW Tasmanian sites).

Table 7.1 - Sites sampled. Listed in order of site name by state.

Site name	Plantation species	Year planted	Treatment structure[A]	Soil classification	No. of trees measured /plot	Previous land use[L]	State	Collaborating Organization[F]
Cussacks	*E. nitens*	1993	3 N x 3 P	Chocolate-Podzolic[B]	9	Open	NSW	SF
Richardsons	*E. nitens*	1993	3 N x 3 P	Krasnozem[B]	9	Open	NSW	SF
Basils	*E. nitens*	1993	5 P	Ferrosol[C]	9	Mixed	Tas.	NET
Boulder	*E. nitens*	1993	5 P	Ferrosol[C]	9	*Pinus radiata*	Tas.	NET
Deacons	*E. nitens*	1992	5 N x 5 P	Ferrosol[C]	9	*Pinus radiata*	Tas.	NET
Dempster	*E. nitens*	1994	2 P	Ferrosol[C]	9	Mixed	Tas.	NET
Exton	*E. nitens*	1992	6 P	Ferrosol[C]	18	Open	Tas.	CSIRO-FFP
Guildford	*E. nitens*	1992	5 P	Ferrosol[C]	9	Open	Tas.	NET
Middlesex	*E. nitens*	1994	2 P	Ferrosol[C]	9	Open	Tas.	NET
Middlesex-7	*E. nitens*	1991	5 N x 5 P	Ferrosol[C]	9	Open	Tas.	NET
Potters	*E. nitens*	1995	4 N x 4 P	Ferrosol[C]	9	Pasture	Tas.	NET
Rabbit Plain	*E. nitens*	1993	5 P	Ferrosol[C]	9	Open	Tas.	NET
Sugarloaf	*E. nitens*	1994	2 P	Ferrosol[C]	9	*Pinus radiata*	Tas.	NET
Wages	*E. nitens*	1991	5 N x 5 P	Ferrosol[C]	9	Mixed	Tas.	NET
Westfield	*E. nitens*	1991	6 P	Kurosol[C]	18	Mixed	Tas.	CSIRO-FFP
Youralla	*E. nitens*	1991	6 P	Ferrosol[C]	18	*Pinus radiata*	Tas.	CSIRO-FFP
Boola	*E. globulus*	1989	2 N x 2 P	Brown Podzolic[B]	36	*Pinus radiata*	Vic.	UM
Glencoe	*E. globulus*	1989	2 N x 2 P	Podzol[B]	36	*Pinus elliotii*	Vic.	UM
Kuark	*E. globulus*	1992	2 N x 2 P	Gradational[D]	n/a	Open	Vic.	CFTT
Maryvale	*E. globulus*	1989	2 N x 2 P	Soloth[B]	36	*Pinus radiata*	Vic.	UM
West Bemm	*E. globulus*	1992	2 N x 2 P	Duplex[D]	n/a	Open	Vic.	CFTT
Beebes	*E. globulus*	1987	8 P	Humus Podzol[B]	18	Open	WA	CSIRO-FFP
Boorara	*E. globulus*	1987	8 P	Gravelly red podzolic[B]	18	Open	WA	CSIRO-FFP
Carpenters	*E. globulus*	1987	8 P	Red earth[B]	18	Open	WA	CSIRO-FFP

[A] Only P treatments applied soon after planting are shown.
[B] Great Soil Group soil classification according to Stace *et al.* (1972).
[C] Australian soil classification according to Isbell (1996).
[D] Soil classification according to Northcote (1979).
[L] *Open* indicates native forest with eucalypt and grass species. *Mixed* indicates native forest with eucalypt and rainforest species.
[F] Abbreviations as follows: CFTT: Centre for Forest Tree Technology, CSIRO-FFP: CSIRO Forestry and Forest Products, NET: North Eucalypt Technologies, SF: State Forests of New South Wales, UM: University of Melbourne.

Figure 7.1 - Map showing locations of Exton, Westfield (Tasmania), and mainland experiments that were sampled.

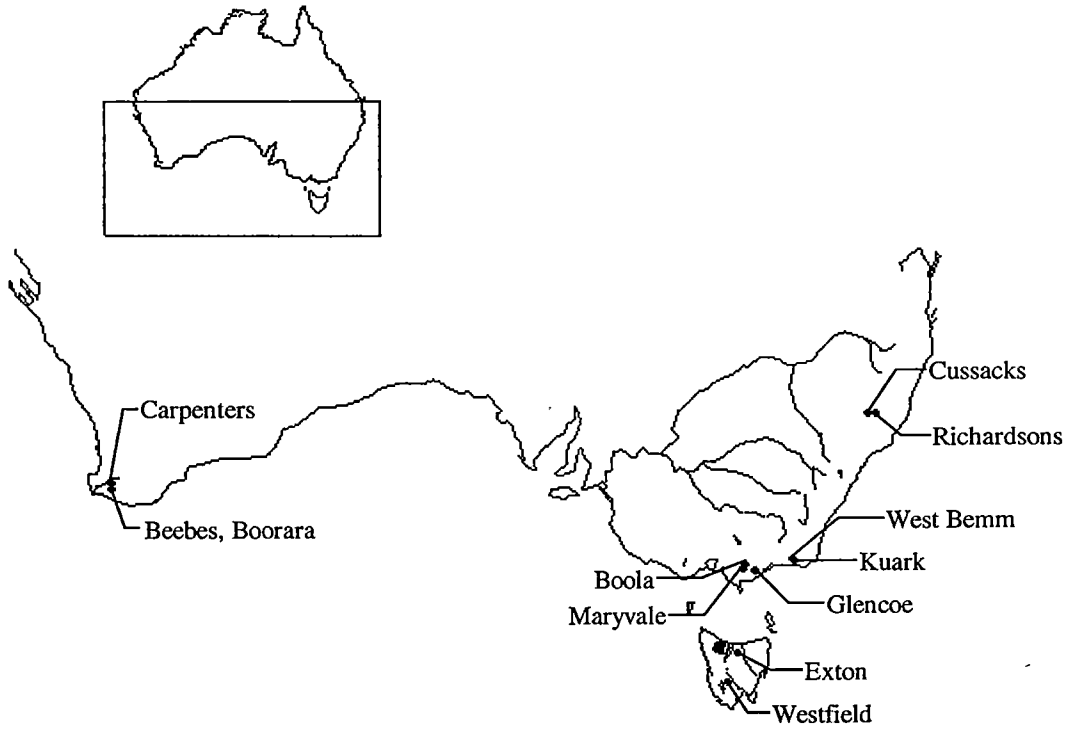
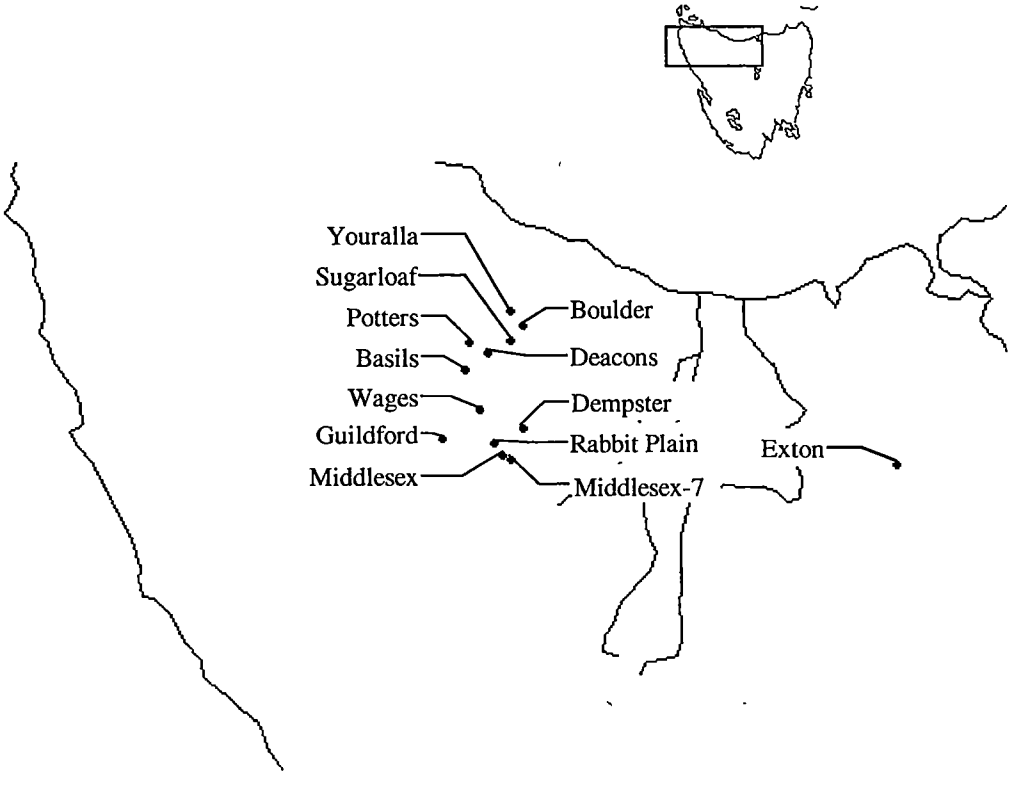


Figure 7.2 - Map showing location of experiments in NW Tasmania.



The Tasmanian sites were sampled in October 1996, and the mainland (Victorian, New South Wales, and West Australian) sites were sampled between November 1997 and March 1998. Soil samples were analysed within 6 weeks of collection.

7.2.2 Soil sampling

Soil samples were taken from treatments where no P fertilizer had been applied. Soil was sampled from four replicates at the Beebes, Boorara and Carpenters sites, and 3 replicates from each of the other sites. The sample location within the plots is described in Section 7.2.2.1, and collection details are described in Section 7.2.2.2.

7.2.2.1 Assessment of sample location within plots

The most effective location for sampling within the control plots was assessed by taking depth samples from a location where soil had been least disturbed by forestry operations ('undisturbed depth' samples), from the 0-10 cm depth range within the mounds, and from the 0-10 cm depth range between the mounds (see Section 7.2.2.2). These samples were taken from each replicate of Boulder, Deacons, Dempster, Middlesex, Middlesex-7, Potters, Rabbit Plain, Sugarloaf, Wages, and Westfield sites, and depths from one location per site were taken from Basils and Youralla. The undisturbed depth samples approximately corresponded to the A, upper B and lower B horizons (Table 7.2). Where there was no distinct boundary between horizons, soil was sampled from 0-10 cm, 10-20 cm, and 20-30 cm depth.

Table 7.2 - Depth ranges sampled at some of the Tasmanian sites.

Depth 1	Depth 2	Depth 3	Sites
0-7 cm	7-14 cm	14-24 cm	Basils
0-5 cm	5-20 cm	20-40 cm	Dempster
0-5 cm	5-15 cm	15-30 cm	Middlesex, Middlesex-7, Rabbit Plain
0-10 cm	10-20 cm	20-30 cm	Boulder, Sugarloaf, Wages, Youralla, Potters, Westfield
0-10 cm	10-20 cm	20-40 cm	Deacons

7.2.2.2 Soil collection details - mound and inter-mound samples

Sites were generally mounded prior to planting. Mounds were 3 - 3.5 m between tops, approximately 180 cm wide, and 50 cm above the original land surface when formed. Soil was collected from within and between the mounds. Twenty subsamples (0-10 cm) were collected and bulked per replicate (along a random transect through the control plot). Soil from the Tasmanian sites was collected with a stainless steel core (25 mm inner diameter). At the mainland sites, samples were taken only from the inter-mound area. A spade was used to collect the depth samples from the undisturbed soil.

7.2.3 Soil analyses

Analyses conducted were $CaCl_2$ P, P sorption curve, Colwell P, Bray No. 2 P, Acid extractable P, and loss on ignition (methods, Section 3.4). Equilibrium buffer capacity, (EK_D), was determined as the slope of the sorption curve at the equilibrium phosphate concentration (EPC).

7.2.4 Growth response to P fertilizer

Most responses to P occur during the first year (see Chapter 2), so growth at 12 months after establishment was used to assess response to P fertilizer. Above-ground dry matter (or volume) was found to be the most sensitive indicator of P deficiency, but only tree heights were measured at some sites at 12 months. An allometric relationship derived from heights (h) and diameters (d) of 12 month old trees at from 5 Tasmanian sites ($R^2 = 0.93$, A. V. La Sala, pers. comm.) was used to convert height to a measure of volume (d^2h). Growth responses had been published for Boola, Maryvale, Glencoe and Carpenters. The former 3 sites were described by Judd *et al.* (1996a), while the latter site was described by Grove *et al.* (1991). Response surfaces for the other experiments have not been published.

Maximum growth response to P fertilizer at each site was determined as the asymptote of a Mitscherlich curve relating growth to P treatment (at a basal level of N fertilizer application). *Relative yield* was defined as growth (dry matter or volume) in the non-P fertilized treatment (control) as a percentage of predicted maximum growth (Equation 7.1).

$$\text{Relative yield (\%)} = \frac{\text{growth in control}}{\text{predicted maximum growth}} \cdot \frac{100}{1} \qquad \text{Equation 7.1}$$

Growth response was defined as the difference between growth in the control and predicted maximum growth in response to P fertilizer, divided by growth in the control and multiplied by 100 (Equation 7.2).

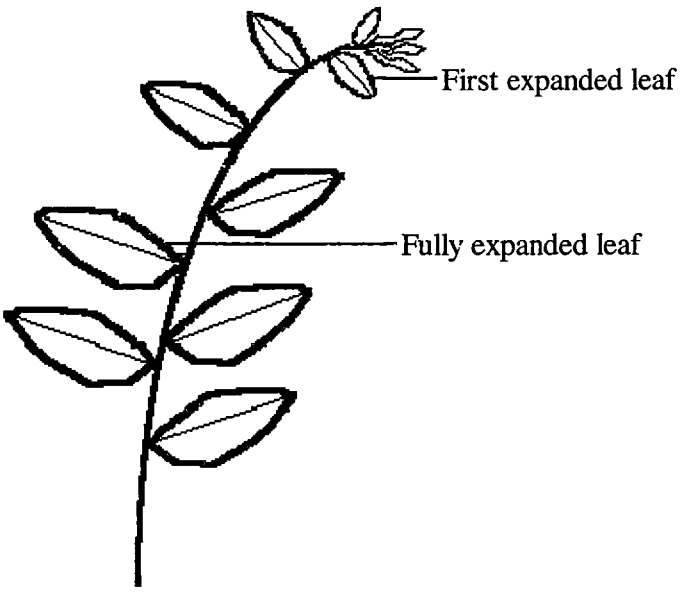
$$\text{Growth response (\%)} = \frac{\text{predicted maximum growth - growth in control}}{\text{growth in control}} \cdot \frac{100}{1} \qquad \text{Equation 7.2}$$

7.2.5 Plant sampling

Foliage samples were collected from all Tasmanian sites (except Westfield and Guildford) in October 1996, when trees were 1-5 years old. Shoot material was taken from 10 randomly selected trees in each of the control and maximum P fertilized treatments. Generally, shoot

material was sampled from the top one-third of the canopy on the northern side of the tree. Specimens of the first newly expanded leaf and first fully expanded leaves (see Figure 7.3) were separated for individual analysis, because they may have had different sensitivity to P deficiency (Smith, 1986).

Figure 7.3 - Diagrammatic representation of a eucalypt shoot, showing positions of the first and fully expanded leaves.



Following drying at 75°C, plant material was ground, digested, and analysed for N and P (method, Section 3.6.4).

7.3 Results

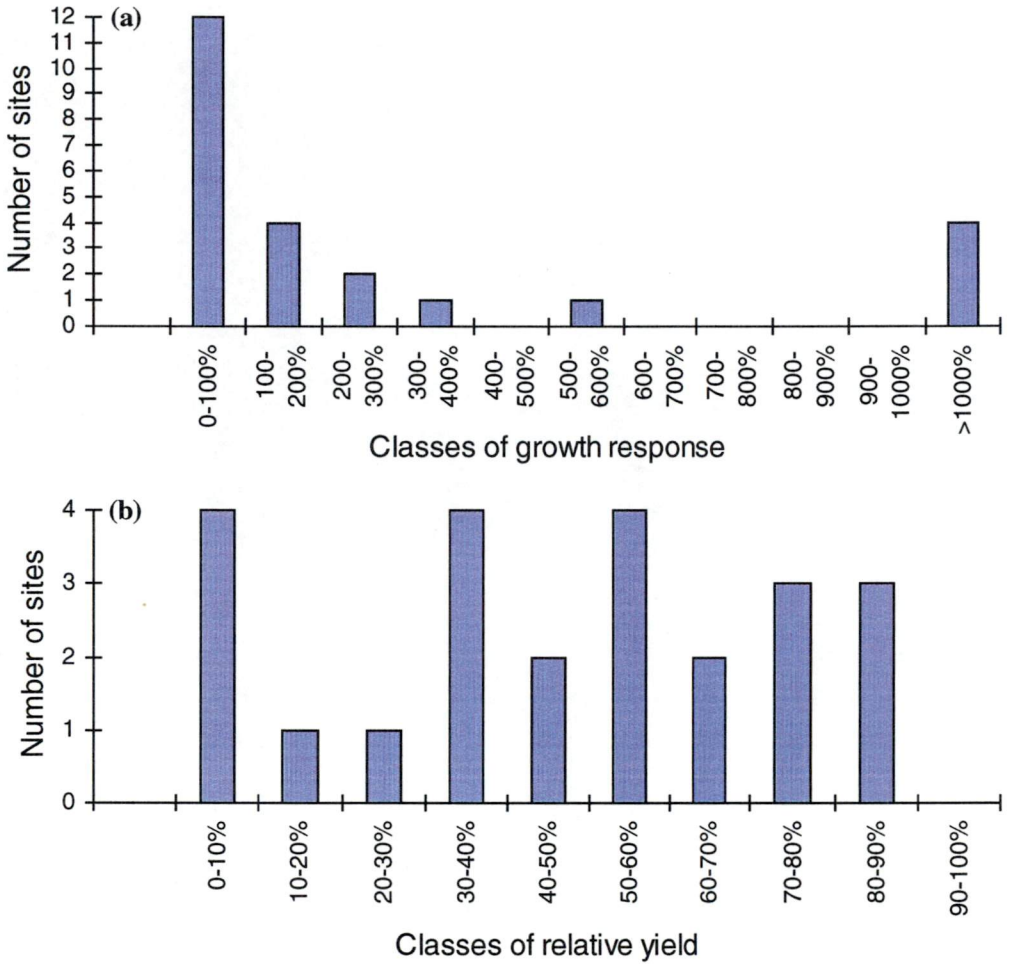
Relative yield in the control treatment ranged from 0.94% (West Bemm) to 88.8% (Middlesex Spur-7), and growth as a percentage increase over the control treatment ranged from 12.6% to 10 591% (Table 7.3). Growth response was unevenly distributed over the range of responses, with the two sites of highest response (West Bemm and Youralla) far above the others (Figure 7.4a). Because there was a much more even distribution of relative

yield (Figure 7.4b), this measure was used in further comparisons.

Table 7.3 - Relative yield and growth response at each site.

Site	Relative yield	Increase over control	Site	Relative yield	Increase over control
Basils-2	55%	82.7%	Kuark	30%	228%
Beebes	6.2%	1513%	Maryvale	63%	58.8%
Boola	54%	83.8%	Middlesex	38%	161%
Boorara	22%	357%	Middlesex Spur-7	89%	12.6%
Boulder	39%	155%	Potters	88%	14.1%
Carpenters	6.3%	1487%	Rabbit Plain	71%	41.7%
Cussacks	53%	88.6%	Richardsons	81%	22.9%
Deacons	55%	83.1%	Sugarloaf	30%	232%
Dempster Ck	43%	131%	Wages	79%	27.2%
Exton	0.94%	10 591%	West Bemm	1.1%	9243%
Glencoe	60%	65.8%	Westfield	44%	129%
Guildford	77 %	29.2%	Youralla	15%	565%

Figure 7.4 - Histogram of response classes at each site, when expressed as growth response (a), and relative yield in the control treatment (b).



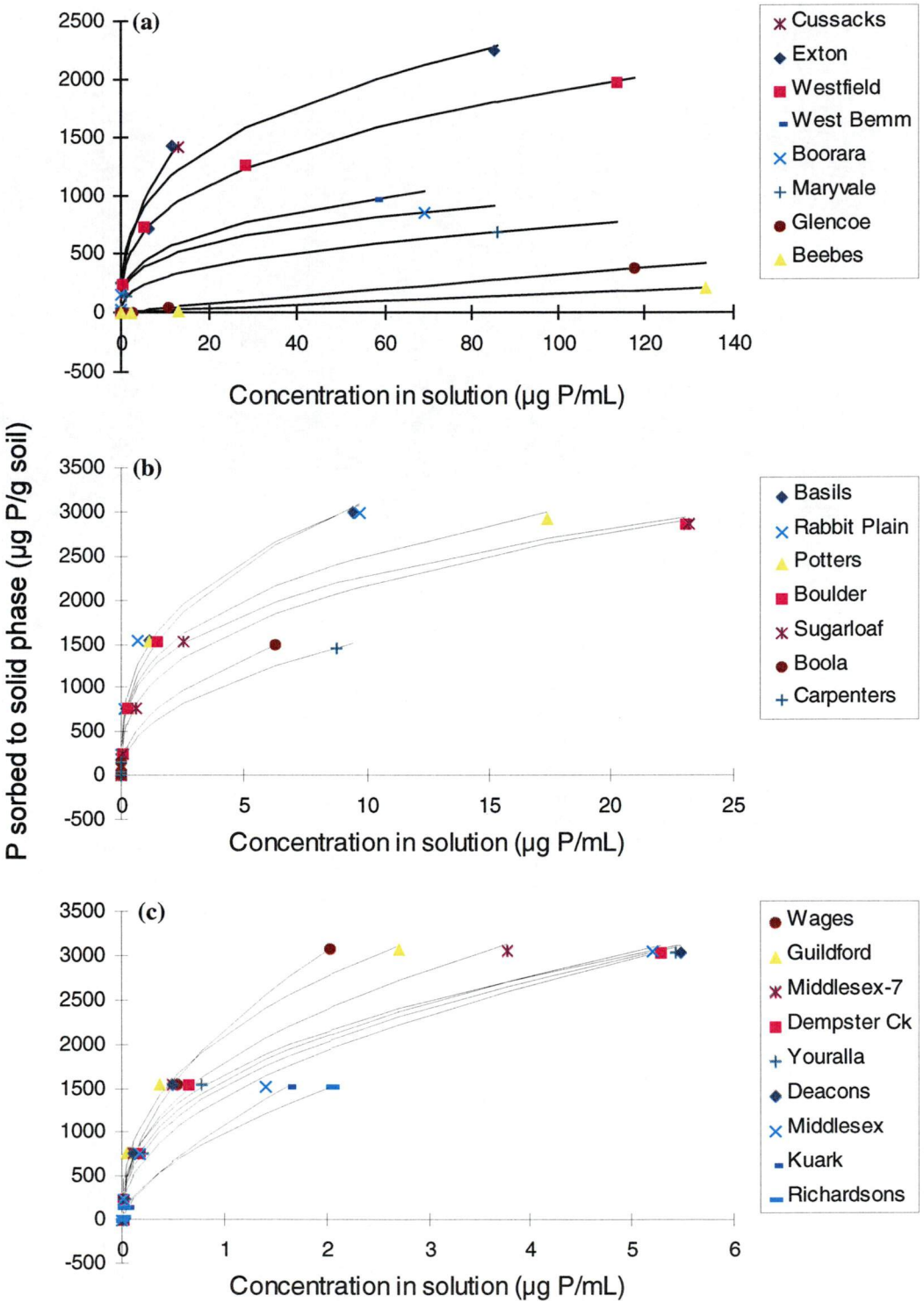
Linear regressions (not shown) were derived to fit sorption data around the equilibrium phosphate concentration (EPC), from which EPC and EK_D were calculated (Table 7.4). The units of EK_D were mL/g. To be consistent with other P intensity indicators (ie. solution P and $CaCl_2$ P), the units of EPC were converted from µg/mL to µM. The value of EPC at the Glencoe site (7.83 µM) was 10-fold higher than the next highest concentration (0.75 µM, at Westfield). Value of EPC in soils other than Glencoe ranged from 0.05 µM to 0.75 µM, with an average of 0.24 µM. Equilibrium P buffer capacity ranged from 6.64 to 48 475 mL/g.

Table 7.4 - Equilibrium phosphate concentration (EPC, μM) and buffer capacity at EPC (EK_D, mL/g).

Site	EPC (μM)	EK_D (mL/g)	Site	EPC (μM)	EK_D (mL/g)
Basils-2	0.26	1.45×10^4	Kuark	0.10	3.30×10^3
Beebes	0.07	1.84×10^3	Maryvale	0.15	2.60×10^3
Boola	0.08	9.36×10^3	Middlesex	0.24	2.48×10^4
Boorara	0.11	3.40×10^3	Middlesex -7	0.43	2.38×10^4
Boulder	0.21	8.59×10^3	Potters	0.34	1.08×10^4
Carpenters	0.14	5.44×10^3	Rabbit Plain	0.28	1.41×10^4
Cussacks	0.17	5.62×10^3	Richardsons	0.18	1.05×10^4
Deacons	0.36	2.42×10^4	Sugarloaf	0.33	7.12×10^3
Dempster Ck	0.37	2.71×10^4	Wages	0.31	3.83×10^4
Exton	0.06	2.83×10^3	West Bemm	0.05	7.01×10^3
Glencoe	7.83	6.64×10^0	Westfield	0.75	9.01×10^2
Guildford	0.22	4.85×10^4	Youralla	0.37	1.69×10^4

Phosphorus sorption curves were derived for each soil. For presentation purposes, the soils were divided into three groups according to their sorption capacity. Sites with relatively low P sorption were: Cussacks, Exton, Westfield, West Bemm, Boorara, Maryvale, Glencoe, and Beebes (Figure 7.5a). Sites with medium P sorption were Basils, Rabbit Plain, Potters, Boulder, Sugarloaf, Boola, and Carpenters (Figure 7.5b), and sites with relatively high P sorption were Wages, Guildford, Middlesex Spur-7, Dempster creek, Youralla, Deacons, Middlesex, Kuark and Richardsons (Figure 7.5c).

Figure 7.5 - Phosphorus sorption curves of soils from each of the experimental sites.



Freundlich coefficients for sorption over the range shown in Figure 7.5 for each soil are shown in Table 7.5. The sites had a wide range of P buffer capacities, from poorly P-sorbing

sands at Beebes and Glencoe, through to highly P-sorbing Ferrosols at most of the Tasmanian sites. The range of the Freundlich *a* coefficient (2.11 - 2153 µg/g) was similar to the range of 11 - 2132 µg/g found by Singh and Gilkes (1991) for 97 soils from south-western Australia. The range of P buffer capacity at EPC (6.64 - 48 475 mL/g) was wider than that observed by Moody *et al.* (1988) for 26 surface soils from Queensland (159-15 702 mL/g).

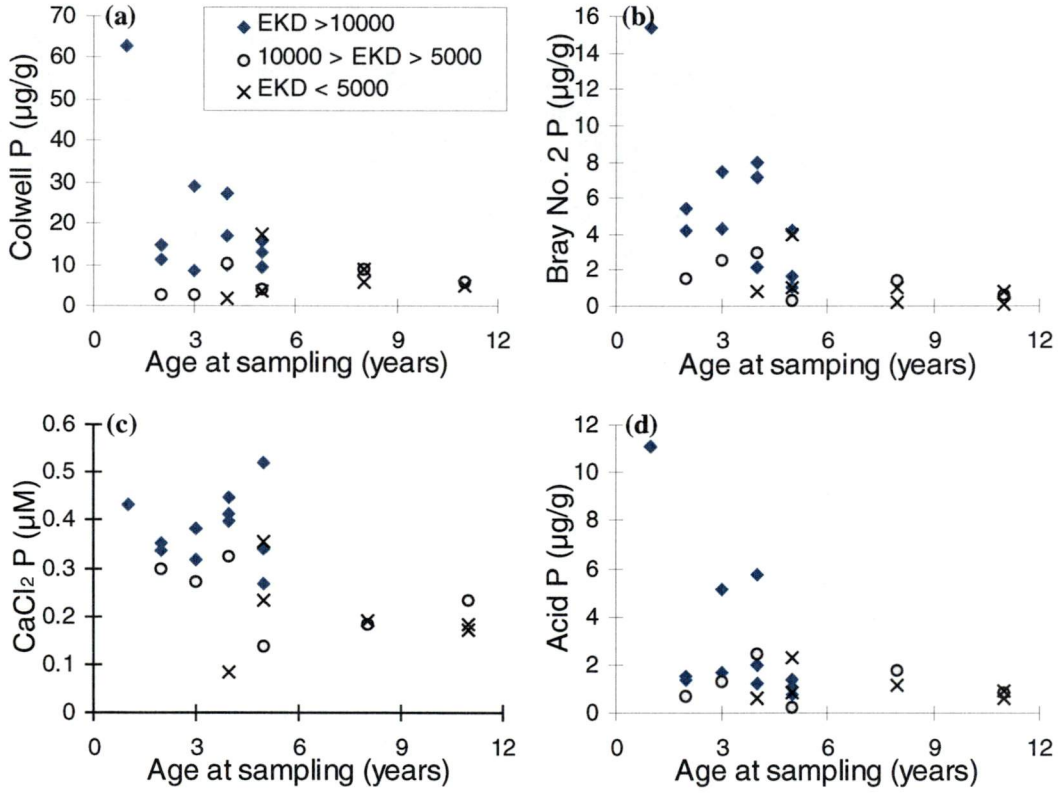
Table 7.5 - Freundlich coefficients for each soil over the concentration range shown in Figure 7.5. Soils in order of increasing EK_D.

Site	Freundlich coeff.		Site	Freundlich coeff.	
	a	*b*		*a*	*b*
Beebes	2.11	1.06	Potters	1203	3.13
Glencoe	4.97	1.10	Rabbit Plain	1433	2.97
Maryvale	125	2.60	Basils-2	1321	2.68
Boorara	230	3.22	Richardsons	987	1.64
West Bemm	253	3.02	Kuark	1088	1.43
Westfield	390	2.90	Middlesex	1398	2.15
Exton	510	2.97	Deacons	1655	2.70
Cussacks	449	2.24	Youralla	1514	2.37
Carpenters	527	2.13	Dempster Ck	1583	2.48
Boola	632	2.15	Middlesex Spur-7	1785	2.38
Sugarloaf	966	2.84	Guildford	2096	2.51
Boulder	1138	3.31	Wages	2153	1.99

The assumption that the time interval between planting and soil sampling caused no change in P analyses was tested by comparing P analyses with experiment age at sampling (Figure 7.6). Linear correlations were not significant between test result and experiment age for

Colwell P (Figure 7.6a), $CaCl_2$ P (Figure 7.6c), and acid extractable P (Figure 7.6d). A significant linear relationship ($P < 0.01$) was found between Bray No. 2 P and time of sampling (Figure 7.6b). There was a confounding effect of P buffer capacity, because all of the highly buffered Ferrosols were younger sites at sampling (less than 6 years old), whereas the older sites had a lower buffer capacity and generally lower test results.

Figure 7.6 - Relationship between test result and age at sampling time.



Correlations between growth response and $CaCl_2$ P were analysed on soil samples from different locations relative to depth and mound position in the control plots of some Tasmanian sites (Section 7.2.2.1, Table 7.6). Soil from between the mounds was most highly correlated with growth and was the only significant correlation when all sites were accounted for.

Because depth samples from the Youralla site had anomalously high $CaCl_2$ P (0.55 - 0.82

μM), these were removed from the analysis. Depth samples were taken from a single location at that site and an unusually high P-microsite was sampled. Without the Youralla data, there was a significant correlation between response to fertilizer and $CaCl_2$ P from soils of undisturbed depth ranges 2 and 3. Response to P fertilizer was not significantly correlated with $CaCl_2$ P from undisturbed A horizon soil (depth 1) either with or without the Youralla data. $CaCl_2$ P from the undisturbed depth 1 sample was significantly correlated with that in the depth 2 sample, and $CaCl_2$ P from the depth 2 sample was significantly correlated with that in the depth 3 sample.

Table 7.6 - Correlation coefficients (r) between growth response and $CaCl_2$ P (μM) from different locations within the control plots of the Tasmanian experiments. For details of sampling locations, see Section 7.2.2.

	Relative yield (% of max)		Mound	Inter-mound	Depth 1	Depth 2
	All sites	Youralla excluded				
0-10 cm Depth						
Mound	0.52	0.50				
Inter-mound	0.85 **	0.86 **	0.64 *			
Undisturbed profile						
Depth 1	-0.03	0.40	-0.32	-0.18		
Depth 2	0.15	0.68 *	-0.1	0.07	0.83 **	
Depth 3	0.45	0.84 **	0	0.39	0.65 *	0.89 **

* $P < 0.05$, ** $P < 0.01$.

For all subsequent analyses, soil samples were taken and analysed from the 0-10 cm depth

between the mounds, because that soil sample gave the highest correlation between $CaCl_2$ P and response to fertilizer.

Relationships between each of the P analyses are shown in Figure 7.7 and Figure 7.8. Glencoe $CaCl_2$ P was not presented in these relationships because of its unusually high value compared with $CaCl_2$ P from the other sites. Significant linear relationships were observed between $CaCl_2$ P and quantity based indicators: with Colwell P ($P < 0.01$, Figure 7.7a), Bray No. 2 P ($P < 0.01$, Figure 7.7b), and Acid extractable P ($P < 0.05$, Figure 7.7c), but all the relationships accounted for less than 45% of the variance. Stronger correlations ($R^2 > 0.77$) were found between the quantity indicators: Colwell P was linearly related to both Bray No. 2 P ($P < 0.01$, Figure 7.8a), and Acid extractable P ($P < 0.01$, Figure 7.8), and an exponential fit best described the relationship between Bray No. 2 P and acid extractable P ($P < 0.01$, Figure 7.8c).

Figure 7.7 - $CaCl_2$ P relationship with Colwell P (a), Bray No. 2 P (b) and Acid extractable P (c) for 23 sites (Glencoe excluded).

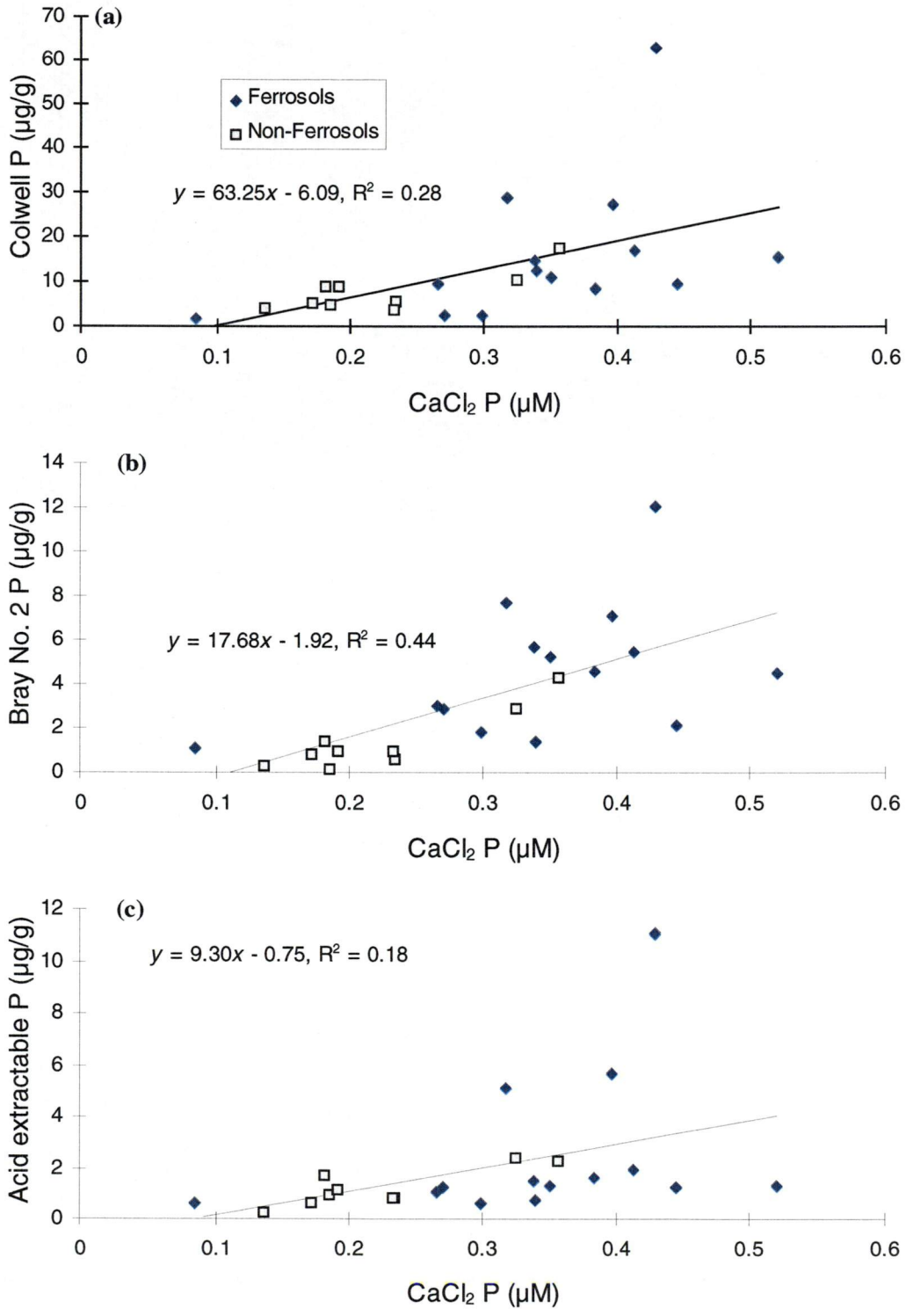
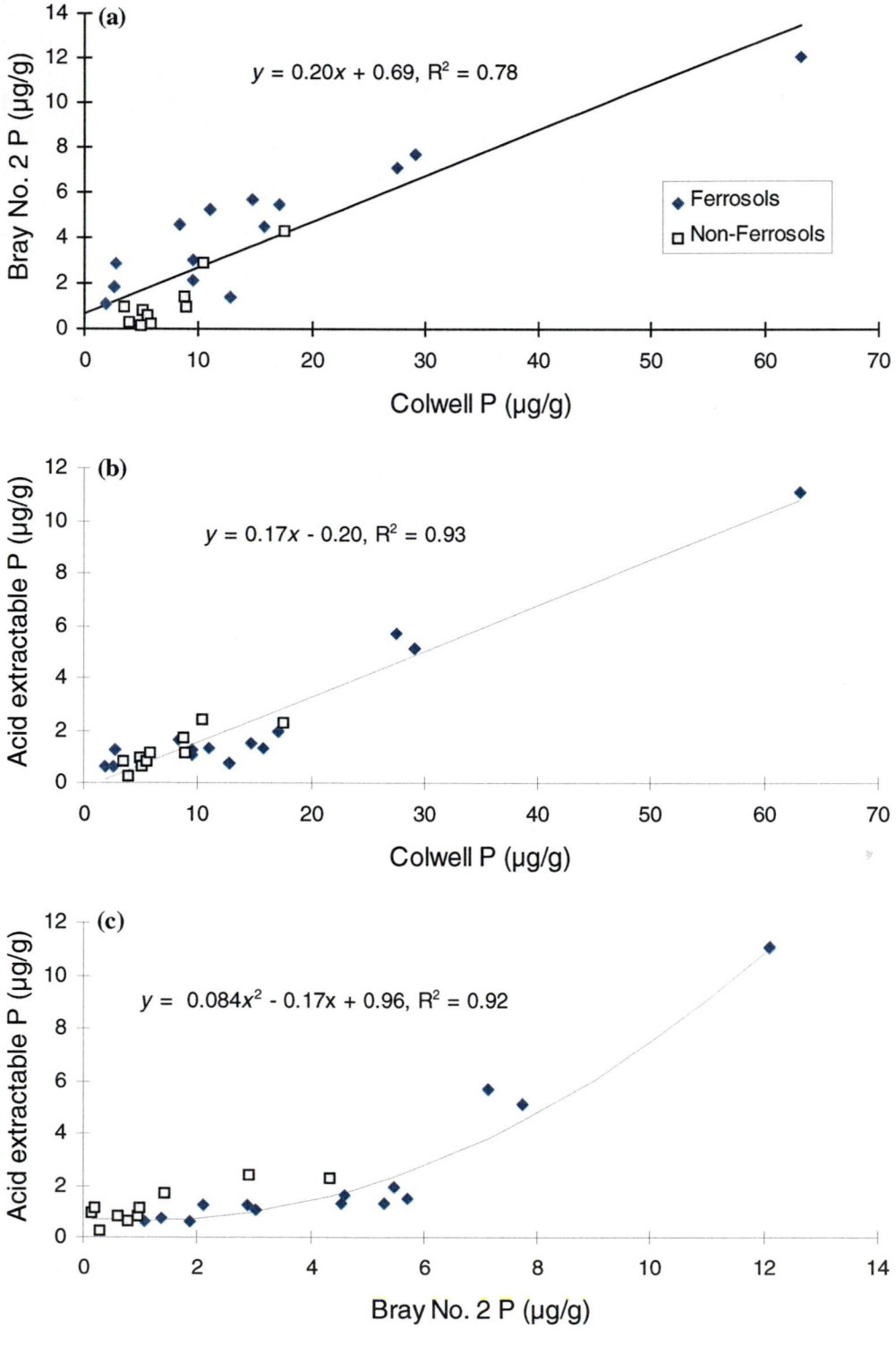


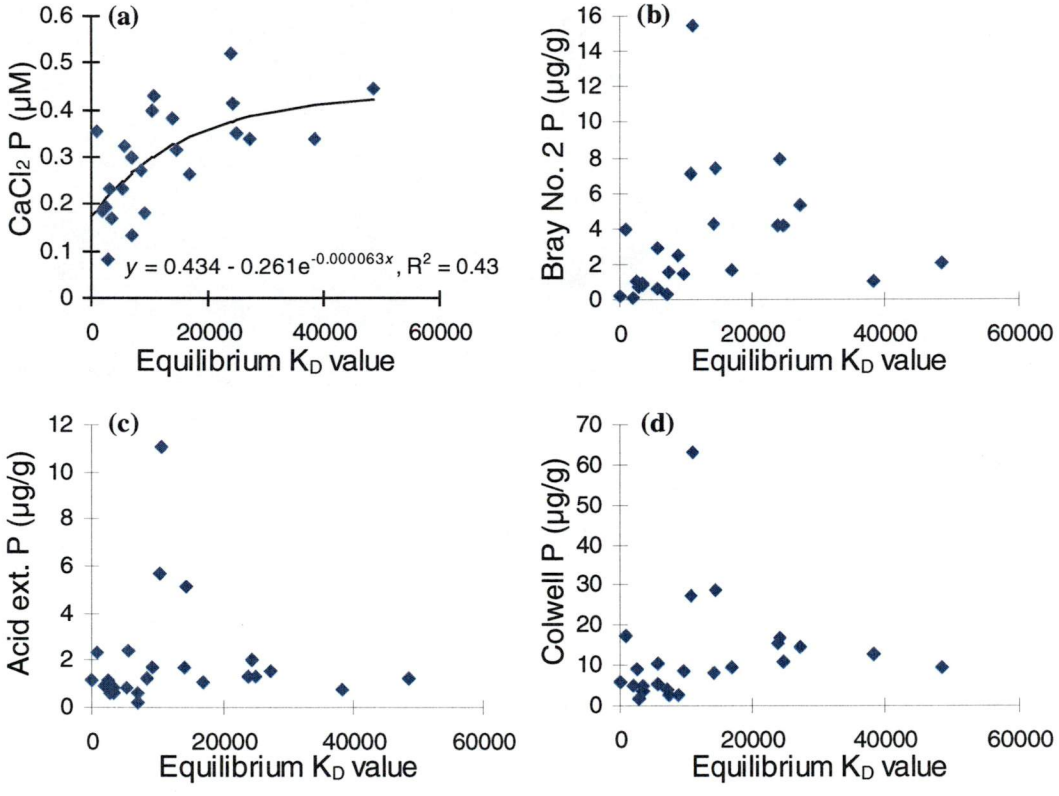
Figure 7.8 - Relationships between Colwell P and Bray No. 2 P (a), Colwell P and Acid extractable P (b), and between Bray No. 2 P and Acid extractable P (c) for the 24 sites.



Equilibrium buffer capacity (EK_D) was significantly correlated with $CaCl_2$ P (Figure 7.9a, P

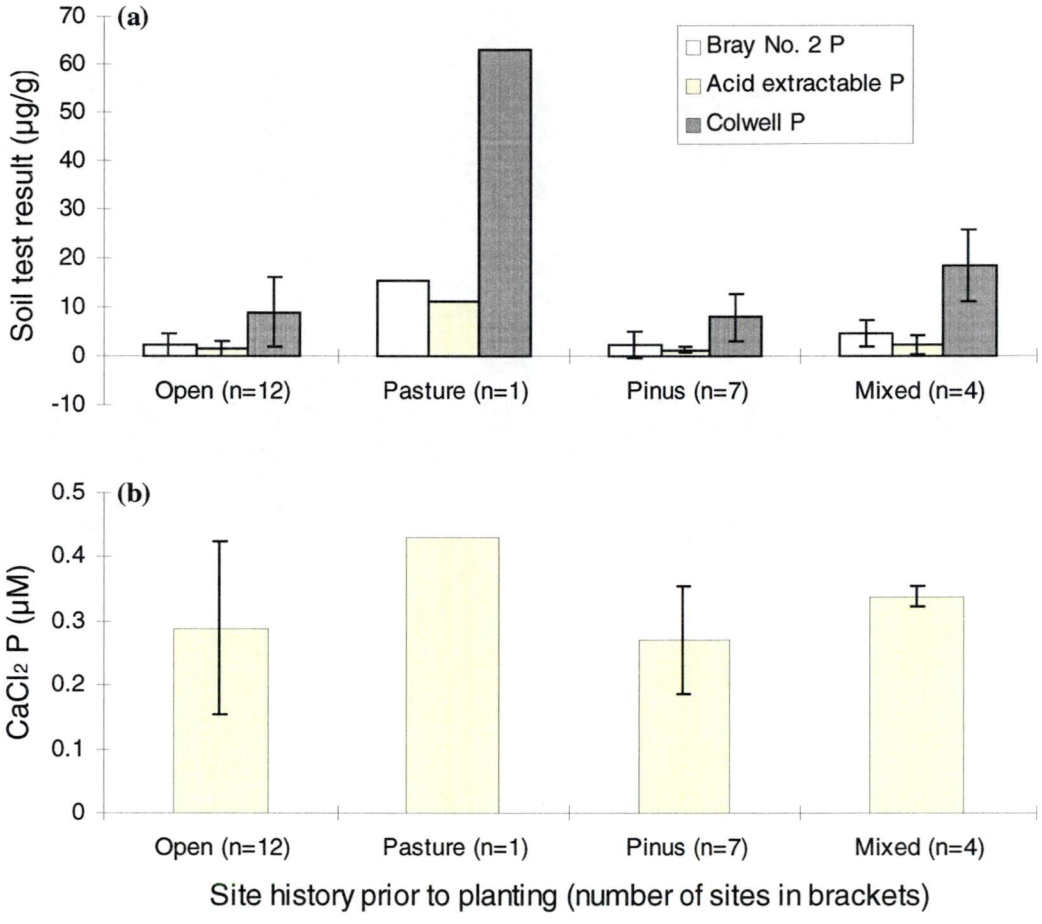
< 0.01), but none of the quantity indicators appeared to be well correlated with EK_D (Figure 7.9b-d)

Figure 7.9 - Relationship between EK_D and $CaCl_2$ P (a), Bray No. 2 P (b), Acid extractable P (c), and Colwell P (d).



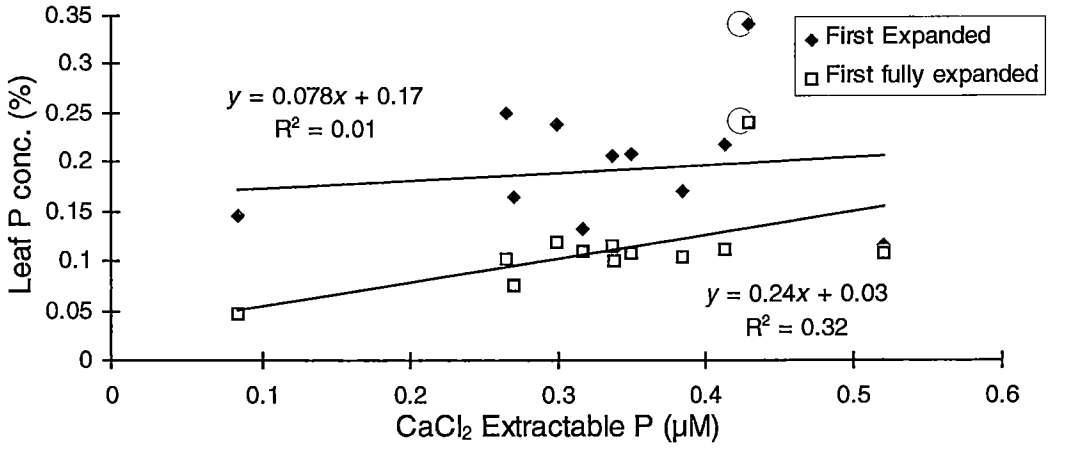
The effect of site history on soil test result was investigated. The numbers of each type of site history were low and unequal, so variation was indicated by standard deviation. Significance was assessed with t-tests. Effects of site history were not significant, except Colwell P from ex-mixed sites was significantly higher than from ex-Pinus or ex-open forest sites. The order of each test was the same: ex-*Pinus* sites ≈ ex-open < ex-mixed < ex-pasture. The ranking remained the same when the comparison was restricted to Ferrosol soil types only, and differences remained non-significant, due to low sample numbers.

Figure 7.10 - Effect of site history on soil analysis result (Error bars represent standard deviation).



Leaf P concentration was assessed at all Tasmanian sites except Guildford and Westfield. The concentration of P in expanded leaves was linearly related to $CaCl_2$ P ($R^2 = 0.32$, $P < 0.05$), but first leaf P concentration was not significantly correlated with $CaCl_2$ P (Figure 7.11). The Potters site (circled) had unusually high leaf P concentrations and when this site was removed from the regression, the significance of the relationship between fully expanded leaf P concentration increased ($P < 0.01$), and was described by the equation: $y = 0.14x + 0.053$, $R^2 = 0.55$. The Potters site may have had high foliage P levels, because it was the only ex-pasture site, it was the youngest at time of sampling (1 year). Additionally, growth may have been limited by temperature at that site (G. K. Holz, pers. comm.).

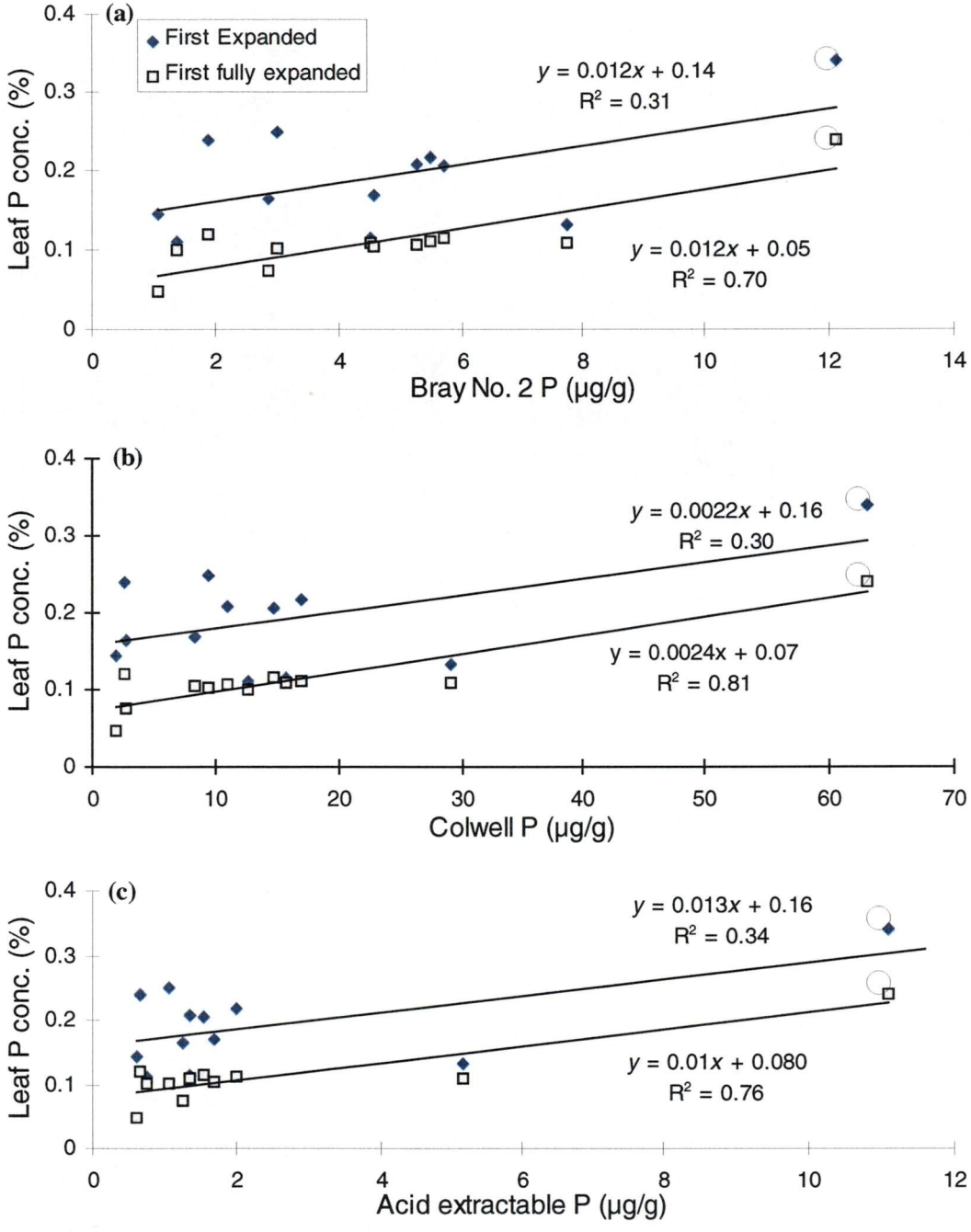
Figure 7.11 - Relationship between leaf P concentration and $CaCl_2$ P at 12 Tasmanian sites.



Significant relationships were also found between leaf P concentration and indicators of soil P quantity, ie. Bray No. 2 P (Figure 7.12a), Colwell P (Figure 7.12b), and Acid extractable P (Figure 7.12c), when all sites were included. Regressions describing the relationships had similar slopes for the first and fully expanded leaves within each type of P analysis, but fully expanded leaves were more highly correlated ($R^2 = 0.70 - 0.81$, $P < 0.01$) with quantity indicators than the first expanded leaves ($R^2 = 0.30 - 0.34$, $P < 0.05$). The Potters site (circled) had the main influence on all regressions, and when that site was excluded, none of the relationships between quantity based indicators and leaf P were significantly correlated.

Average first expanded leaf P concentration was 0.19%, and average fully expanded leaf P concentration was 0.11%.

Figure 7.12 - Relationship between leaf P concentration and indicators of soil P quantity at 12 Tasmanian sites.



Significant Mitscherlich relationships were found between all soil P quantity indicators and growth response when all points were considered (Figure 7.13). Acid extractable P described 43% of the variation in relative yield ($P < 0.05$, Figure 7.13a), Colwell P described 48% ($P <$

0.05, Figure 7.13b), and Bray No. 2 P described 35% ($P < 0.05$, Figure 7.13c). Critical levels (ie. that predicted at 90% of maximum growth) of each of the quantity indicators were: 2.37, 20.57, and 5.43 µg/g for Acid extractable P, Colwell P, and Bray No. 2 P, respectively. However, the asymptote of those regressions did not occur at a relative yield of 100%, but was between 71% and 76%, due to variability in P test data. A Mitscherlich model was also fitted to the lower boundary of the points, because maximum growth in response to P fertilizer may be lower than possible, if trees were limited by some factor other than P at each site. This effect would cause a lower-than-calculated relative yield. Hence the boundary line was fitted through the lower points. Critical levels (ie. for 90% of maximum growth) calculated from the boundary lines were 16.99, 70.63, and 23.74 µg/g for the Acid extractable, Colwell, and Bray No. 2 P, respectively. The range of critical concentrations (ie. between the two methods of calculation of critical concentration) found for each P analysis was also similar to the range of observed maximum growth at individual sites.

Inclusion of indicators of buffer capacity (EK_D, Freundlich or Langmuir coefficients) in multiple linear regressions with P quantity indicators did not improve the significance of any relationships with response to soil P (data not shown).

No trend was found between growth response and EK_D (Figure 7.14a), but there was a highly significant growth response to $CaCl_2$ P (Figure 7.14b). With the exception of the Boola, Maryvale, and Glencoe sites, $CaCl_2$ P described 81% of the variance in growth response. When Boola and Maryvale were included in the regression, the equation was: $y = 2.02x - 0.15$ ($R^2 = 0.63$). Glencoe was not included in the regressions or shown on the graph, because it had an inexplicably high $CaCl_2$ P concentration (4.32 µM, 63% relative growth), which was an order of magnitude greater than found at the other sites.

The critical concentration of $CaCl_2$ P required for 90% of maximum growth predicted by the linear regression was 0.50 µM.

Figure 7.13 - Relationship between relative yield and quantity-based indicators of P availability.

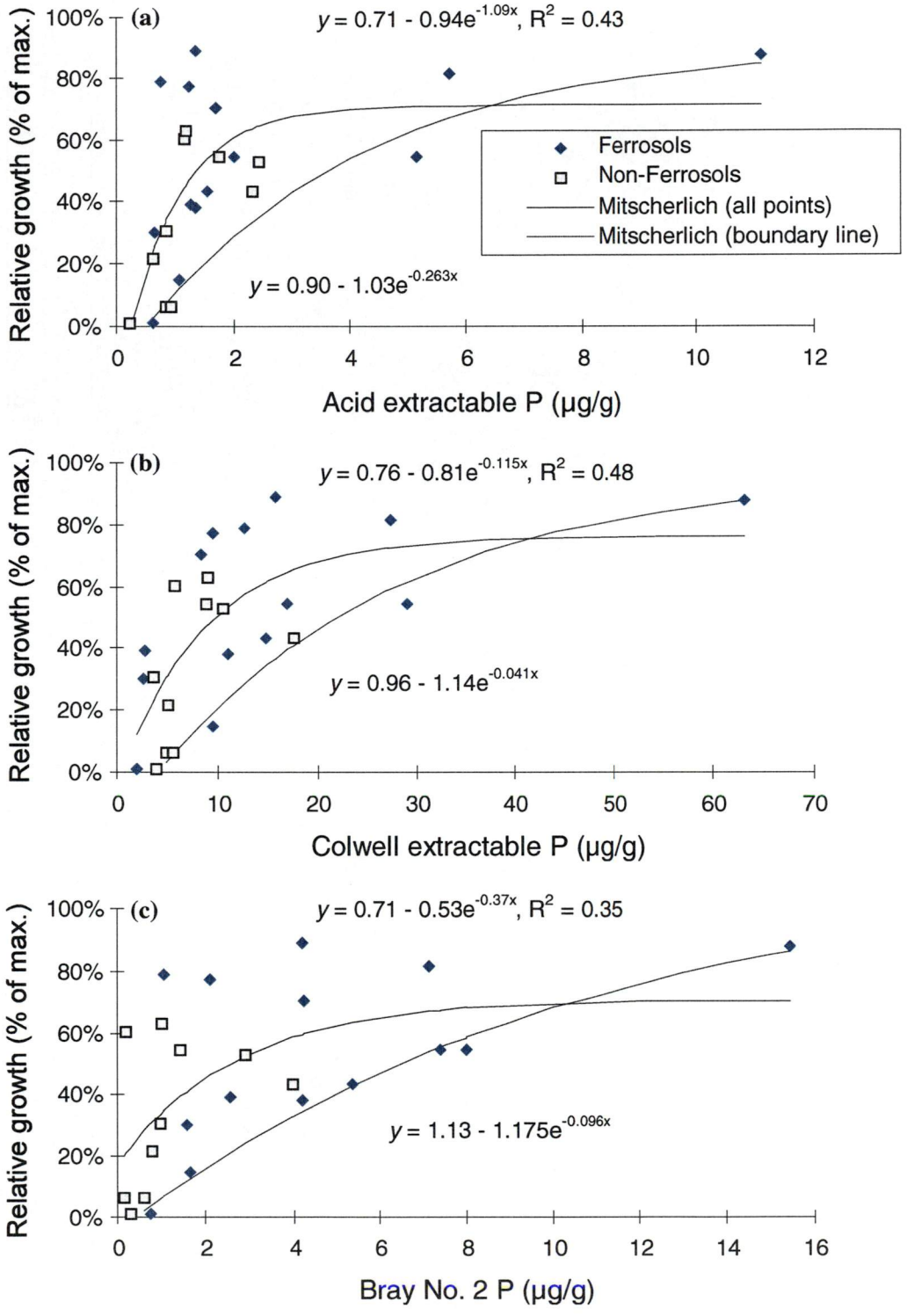
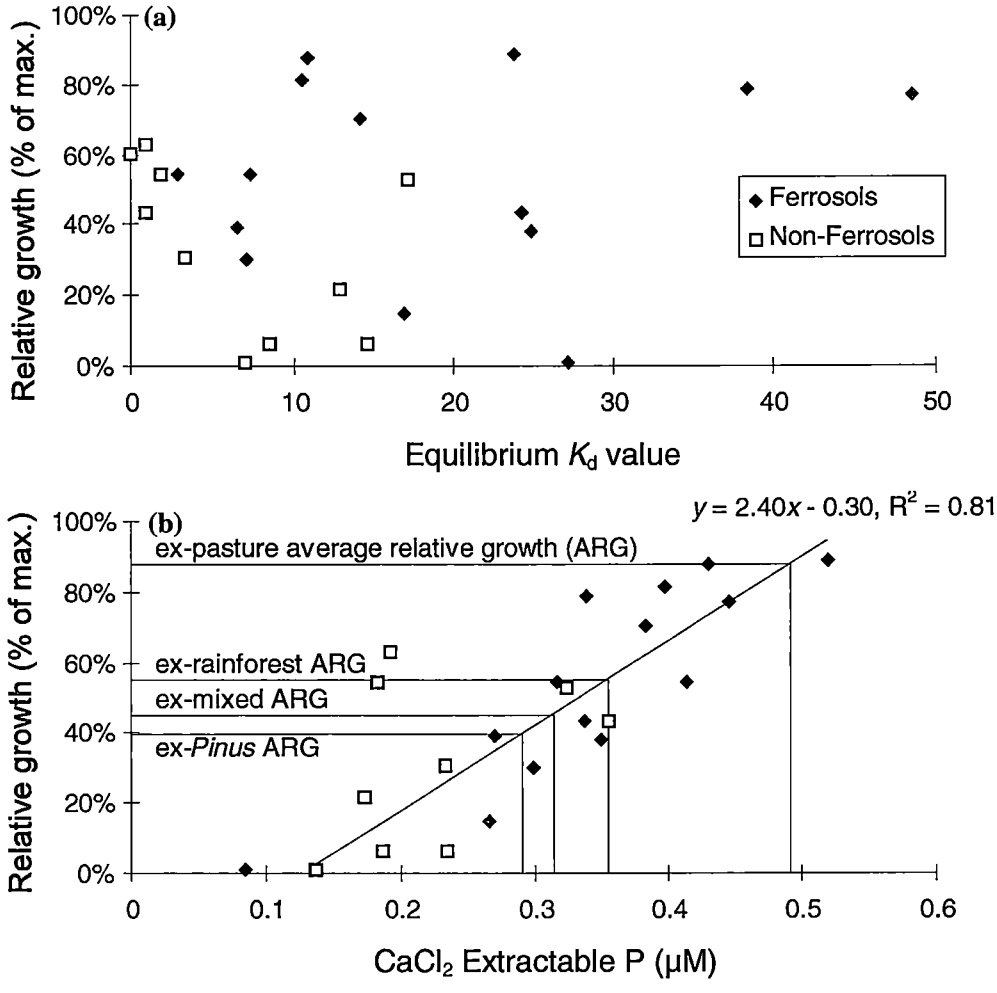
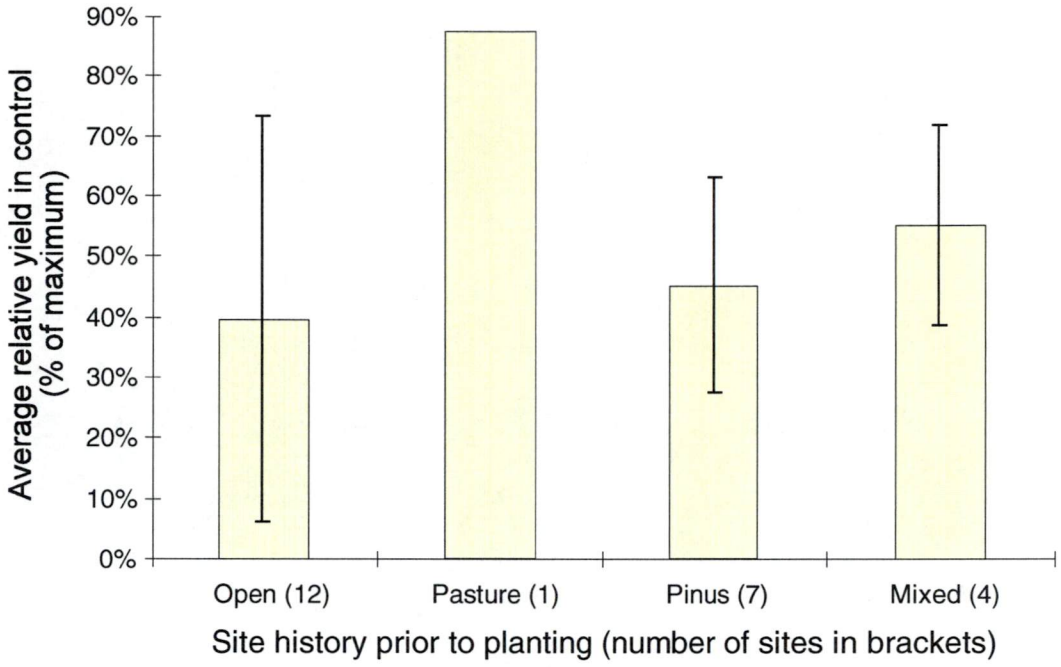


Figure 7.14 - Relative yield in relation to EK_D (a) and $CaCl_2$ P (b).



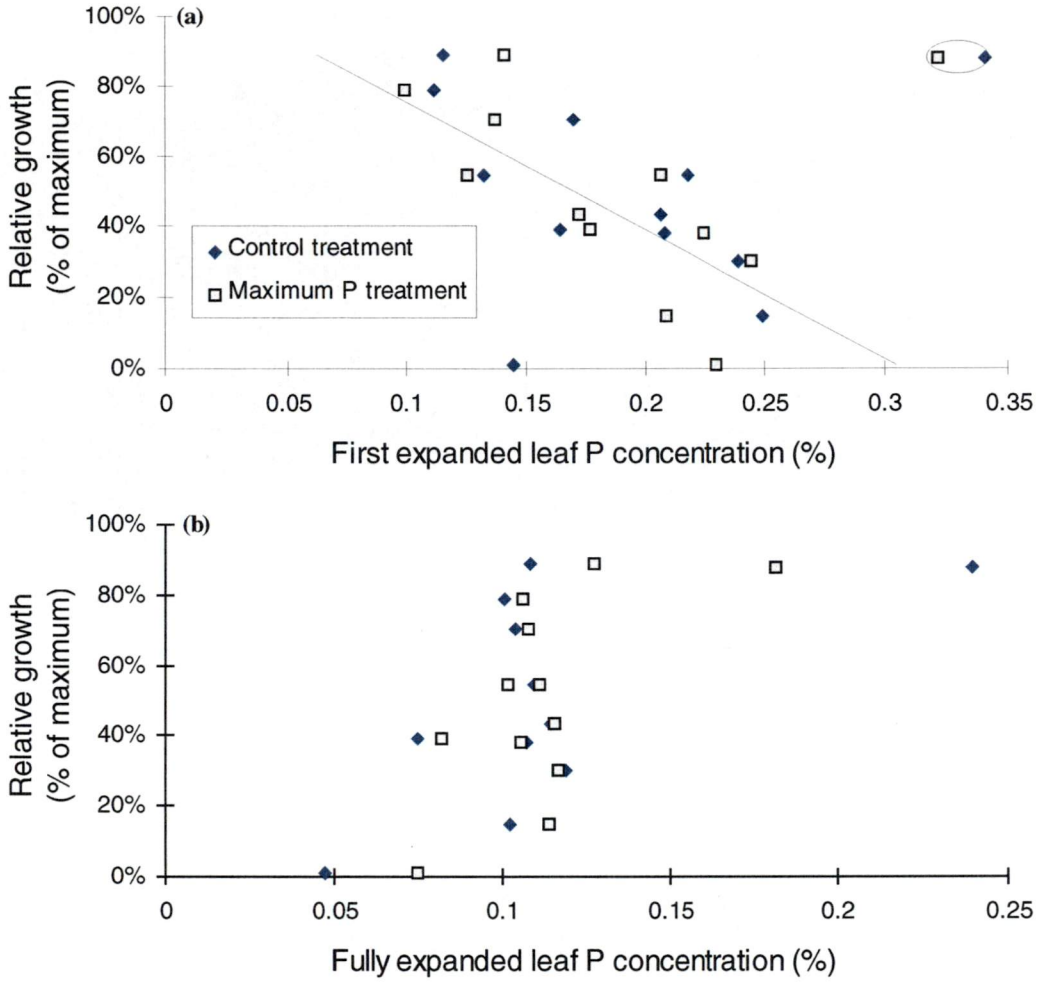
Site history had no significant effect on relative growth at each site (Figure 7.15). The trend was similar to that for soil P indicators (Figure 7.10), but relative yield in open native forest was less than relative yield in ex-*Pinus* plantations.

Figure 7.15 - Average relative yield in control treatments relationship with site history (Error bars show standard deviation).



Overall, there was no significant relationship between relative growth and leaf P concentration (Figure 7.16a, Figure 7.16b), but when the Potters site (circled) was omitted from the analysis, a significant regression ($y = -3.63x + 1.12$, $R^2 = 0.44$) was obtained for the first expanded leaf P concentration. There was no significant treatment effect on concentration in either the first or fully expanded leaves, indicating that growth compensated to maintain similar internal nutrient levels at most sites, irrespective of P availability.

Figure 7.16 - Relative growth response relationship to first expanded leaf P concentration (a), and fully expanded leaf P concentration (b) at 10 Tasmanian sites.



7.4 Discussion

Weak trends in soil test value over time were evident when all sites were considered, but this was also affected by soil buffer capacity and site history. Potters was the youngest site at sampling (1 year), and the only ex-pasture site (and hence had a history of P fertilization), so high levels of labile soil P at that site were not surprising. The assumption of minimal change in P test result over time was supported by the lack of relationship between age at sampling and test results (Figure 7.6), and by Flinn *et al.* (1982), who found no significant change in Bray No. 2 P over 3 years at one site under *Pinus radiata*.

The lower P-sorbing soils (Figure 7.5a) had similar sorption properties to the soils investigated by Ballard and Fiskell (1974), and Fox and Kamprath (1970), see Figure 2.5 and Figure 2.6. A significant increase was observed in $CaCl_2$ P with increasing P buffer capacity at EPC (Figure 7.9a), but no relationships were found between P buffer capacity and quantity indicators in the current study (Figure 7.9b-d). Both Dalal and Hallsworth (1977, 20 soils), and Moody *et al.* (1988, 26 soils) found little correlation between P buffer capacity indicators and Colwell P for soils with a wide range of phosphate levels. Dalal and Hallsworth (1977) also found no relationship between potential buffering capacity and $CaCl_2$ P, and Moody *et al.* (1988) found an approximately inverse relationship between $CaCl_2$ P and equilibrium buffer capacity. The positive correlation found in the current study may have been because most of the soils (14 of 24) were Ferrosols, so intensity is likely to be proportional to quantity (Holford and Mattingly 1976). Another factor influencing the negative relationship found by Moody *et al.* (1988) was that the soils in that study were agricultural, and hence some had been highly P fertilized. Fertilization causes a shift up the sorption curve, thus increasing solution concentration but reducing equilibrium buffer capacity (Holford and Mattingly 1976). In the current study, most of the soils were unfertilized, so they were at the bottom of the sorption curves (where EK_D is high). The pasture soil (Potters) had high P test values, but a relatively low EK_D, which was probably also an effect of past fertilization causing a shift up the sorption curve, reducing EK_D.

The value of $CaCl_2$ P from soil between the mounds gave best correlations with growth, closely followed by little disturbed soil from deeper in the B horizon (Depth 3, Table 7.6). The poor (non-significant) correlation between growth and $CaCl_2$ P from within the mound may have been due to high soil heterogeneity in the mound after cultivation, which reduced the correlation below significance. However, the value of $CaCl_2$ P from soil within the mound was significantly correlated with $CaCl_2$ P from soil between the mounds. The correlation between $CaCl_2$ P between the mounds and $CaCl_2$ P from undisturbed depth samples increased with depth, and became highly significant with the third depth samples

(which were mostly 20-30 cm, see Table 7.2). The high correlations with lower depth samples from undisturbed profiles suggested that most of the A horizon had been lost from between the mounds.

Significant correlations were found between all P quantity and intensity indicators, but no relationships between P quantity indicators and EK_D were found (Figure 7.7, Figure 7.8). Regressions between P intensity (indicated by $CaCl_2$ P) and P quantity indicators were much poorer (Figure 7.7) than those regressions between P quantity indicators only (Figure 7.8). Dalal and Hallsworth (1977) also found better correlations between indicators of P quantity than between indicators of P quantity and intensity in 20 soils with a wide range of phosphate characteristics.

The only inconsistent $CaCl_2$ P test result was at Glencoe, which had a $CaCl_2$ P 10-fold higher than at any other site. Values of $CaCl_2$ P were similar from all 3 replicates at the Glencoe site and a high EPC was observed in the sorption curve analyses (Table 7.4). Other P extracts at the Glencoe site were within the normal range. The reason for the unusually high value of $CaCl_2$ P is not known, but evidence above indicated that it was not a sampling error (eg. sampled from a fertilized treatment), or an error that arose during analysis. Soil from the Glencoe site had the second-lowest sorption of the sites investigated, so P intensity would be poorly buffered against any inputs. It was possible that P release from breakdown of organic matter may have caused the high $CaCl_2$ P. Canopy closure had occurred at that site, which would account for high levels of organic matter (ie. litter), but a similar situation should have occurred at the Beebes site, which had the lowest P sorption, and was also well advanced into the rotation (11 years). Phosphorus leaching at Glencoe could be expected after heavy rain, due to low buffer capacity and high $CaCl_2$ P levels.

Of the analyses investigated, Colwell extracted most P from the soil, followed by Bray No. 2, then Acid. As expected, all of the quantity analyses extracted more P than $CaCl_2$ (see Chapter 6, Moody *et al.* 1988).

There was a weak trend between previous land use and P test result, with low P levels (and high response) at ex-*Pinus* sites, and high P levels (and low response) at an ex-pasture site (Figure 7.10, Figure 7.15). Wang *et al.* (1996a) found the same ranking of sites with indices of nitrogen (total N, anaerobically mineralizable N, hot KCl N) on Ferrosols under eucalypt plantations of north-western Tasmania, and Birk (1993) found that *Pinus radiata* growth was greater on an ex-pasture site than on an adjacent ex-native forest site. The high degree of variability was indicative of the wide range of sites used in the survey.

The concentration of P in first expanded leaves was more variable than in fully expanded leaves (as indicated by lower correlations in Figure 7.11 and Figure 7.12). Older leaves have been suggested as the most appropriate place to sample foliage in eucalypts for an indication of P availability (Dell *et al.* 1995) because phosphorus is highly mobile in the plant, and is readily translocated from older to younger tissues in the event of deficiency. In this study, it was found that fully expanded leaves were reasonably constant in their P levels (Figure 7.16b), except for those from the Potters and Exton sites, which had low and high response to P fertilizer, respectively. Hence fully expanded leaves would not generally be useful for indicating P status. However, the first expanded leaves were significantly (although negatively) correlated with relative growth (Figure 7.16a). Further research is required to elucidate this relationship more fully, but first expanded leaves may be more useful for indicating P status on most sites. The negative correlation obtained between relative growth response and concentration of P in the first expanded leaves (Figure 7.16a) indicated that plants growing poorly had high P levels in the rapidly growing shoot tips. This may have been a dilution effect (ie. available P was diverted to a greater quantity of new foliage at sites with higher absolute growth Figure 7.16b), but again, more research would be required to better elucidate this relationship.

The concentration of P in fully expanded leaves at 10 Tasmanian sites (0.07 - 0.13%) was within the typical range for plantation eucalypts of 0.05 to 0.15% found by Judd *et al.*

(1996b), who reviewed more than 500 published reports of P concentrations in eucalypt foliage. The two sites out of this typical range were Exton (just below, at 0.047% P), and Potters (0.24% P) which also had the lowest, and third highest $CaCl_2$ P values, respectively. Fully expanded leaf P concentrations at the Potters site were significantly higher than at any other site. When leaf P concentrations at Potters were omitted from regressions with P availability indices, all correlations between P quantity indicators and leaf P concentration were reduced below significance. Only $CaCl_2$ P was significantly (and highly) correlated with leaf P concentration when the Potters site was omitted. Except under conditions of extreme P deficiency (eg. Exton), or sufficiency (eg. Potters), *E. nitens* trees in Tasmania adjusted their growth rates to maintain an approximately constant internal P concentration of 0.11%, even over a wide range of ages (2-5 years), and growth conditions.

Response to P fertilizer was investigated on several soil types, and it is generally accepted that quantity-based P analyses are specific to soil type (Holford 1997). Even within the Ferrosol soil type, there was high variability in response to P quantity indicators. Although variability was high, significant asymptotic relationships were obtained between quantity-based soil P analyses and relative growth in control treatments (Figure 7.13). When all sites were accounted for, critical levels for the Acid, Colwell and Bray No. 2 P were 2.37, 20.57, and 5.43 µg/g, respectively. However, critical values were much higher when boundary lines were considered (16.99, 70.63, 23.74 µg/g for the Acid, Colwell, and Bray No. 2 P, respectively). The critical values suggested by the boundary line analysis were higher than would generally be expected for agricultural crops (eg Reuter *et al.* 1995), due to high variability of the Colwell analysis. Hence, critical concentrations that are applicable in the field were difficult to define. Another problem was that there were few sites with little response to P fertilizer. If more sites of higher fertility had been included, then the critical concentration may have been better defined.

The highly significant relationship between $CaCl_2$ P and relative growth at 1 year of age

(Figure 7.14) was by far the best correlation, indicating that $CaCl_2$ P would be a useful indicator of P deficiency at new sites. Alone, $CaCl_2$ P was also better correlated with relative growth than combinations of soil P quantity, intensity or buffer power.

Boola and Maryvale had higher relative growth rates than other sites with similar $CaCl_2$ P levels. The cause of this anomaly may have been reduced maximum growth due to the relatively low rate of P applied soon (2 months) after planting (50 kg/ha). No further response to P fertilizer was obtained at later fertilizations, even though higher rates of P were applied at those times (up to 150 kg/ha extra). Greater maximum growth may have been obtained at those sites if higher rates had been applied soon after planting. Schönau and Herbert (1989) cited several examples where later fertilization was less effective than fertilization soon after planting. Another factor that may have affected the relationship at those sites was that they were 3 of the 8 sites planted with *E. globulus*. Not enough data was collected for that species to fully elucidate trends, but *E. globulus* may have a lower critical concentration than *E. nitens*.

Indicators of soil P quantity have been well correlated with P deficiency in *Pinus* plantations. For example, Hopmans *et al.* (1978) found significant correlations between Bray No. 2 P and growth of *Pinus radiata* at 10 sites on one soil type in Victoria. Ballard (1974) found that Bray and Olsen P were well correlated with growth response of *Pinus radiata* at 16 New Zealand sites. Ballard and Pritchett (1975) found that field growth of *Pinus elliottii* in fertilized plots was well correlated with P intensity during the first year, but the significance of the relationship declined with stand age. Only quantity-based P indices (eg. Bray No. 2 and Colwell P) were significantly correlated with relative growth and P uptake at 3 and 5 years of age. This approach was further refined (Skinner *et al.* 1991) to include a measure of P buffering capacity by sequentially extracting P using the Bray reagent.

Integrating both soil P buffering and quantity indicators has increased correlations between test result and growth of ryegrass (Gunary and Sutton 1967, Holford and Mattingly 1976) and

wheat (Dalal and Hallsworth 1976, Holford and Cullis 1985). In contrast to these results, inclusion of buffer capacity measures in regression analysis with soil P quantity indicators (Bray No. 2, Colwell, Acid) in the current study did not increase the significance of the relationship between test result and relative growth response, suggesting that eucalypts utilize the reserves of soil P differently to ryegrass and wheat. Other crops for which growth is well correlated with soil P intensity include soybeans (Moody *et al.* 1983), subterranean clover (Dear *et al.* 1992), maize, soybeans, groundnuts and potatoes (Fox 1981). The optimum EPC found by Moody *et al.* (1983) for soybeans was approximately 0.4 μM, while the optimum solution concentration for growth of subterranean clover was approximately 3 μM (Dear *et al.* 1992, Ozanne and Shaw 1967). The optimum $CaCl_2$ concentration found for eucalypts in the current experiment (0.50 μM) was similar to that found for soybeans by Moody *et al.* (1983).

The critical Colwell P for wheat in West Australian soils was between 10 and 100 μg/g (depending on soil type), with an average at about 40 μg/g (Bolland *et al.* 1994). Reuter *et al.* (1995) reviewed the results of more than 580 field experiments in South Australia, and found Colwell P to be a useful indicator of response to P fertilizer in a range of crops. Typical Colwell P values for 90% of maximum growth were 21 ± 1 (s.e.) μg/g for wheat (306 experiments), 18 ± 3 μg/g for barley (41 experiments), 13 ± 6 μg/g for potatoes on low P-sorption soils, and 46 ± 10 μg/g for potatoes on medium to high P-sorption soils. The critical value of Colwell P for eucalypts in this study was between 20.57 and 70.63 μg/g, which was similar to (or higher than) the range of the values quoted above. The highly P sorbing Ferrosols influenced those regressions, which may have elevated the critical Colwell P to a higher level than if it was measured on a group of soils with low P sorption, as indicated with the potato comparison above (Reuter *et al.* 1995). Moody *et al.* (1997) found a critical Colwell P for maize of 20-32 μg/g (depending on model fit of the data) in 17 experiments on highly P-sorbing Ferrosols. Critical soil test values for optimal growth have rarely been

quoted in forestry, despite significant correlations between soil P indicators and growth (Ballard and Pritchett 1975, Kadeba and Boyle 1978, Hopmans *et al.* 1978). Ballard (1974) found that 12 μg/g Bray No. 2 P was optimal for growth of *Pinus radiata* in 16 New Zealand experiments, which was similar to the range of 5.43 - 24 μg/g found in these experiments. The time of shaking during the Bray No. 2 extract procedure is not standardised, and significantly affects the amount of P extracted, especially between 40 s and 10 min. (Stewart *et al.* 1990). However, Bray No. 2 test results were comparable between Ballard (1974) and this study, because the same procedure was followed (Blakemore *et al.* 1987).

In conclusion, $CaCl_2$ P was an excellent indicator of response to P fertilizer in the first year of growth of *E. nitens* and *E. globulus* plantations established on a wide range of soils. Three sites from Victoria did not fit this relationship, which may have been due to fertilization régime, site age at measurement, site characteristics, or a species difference between *E. nitens* and *E. globulus*. Colwell P, Acid extractable P, and Bray No. 2 P, were also correlated with response to P fertilizer, but the correlations were poorer than with $CaCl_2$ P. Critical values of the P quantity analyses were difficult to define, and hence these analyses would not be practical to use in the field situation.

8. Modeling P uptake and deficiency in *Eucalyptus* seedlings.

8.1 Introduction

Commonly used soil analyses of P availability attempt to extract an amount of P that is related to the pool of P available to plants during a crop cycle (Fox, 1981). However, these soil analyses require soil- climate- and crop specific calibrations (Holford 1997, Moody *et al.* 1988, Cox 1994), which limits their application to a wider range of conditions without further calibration. In the case of P particularly, the relationship between common availability indicators and plant growth is dependent on interactions among soil, plants and environment (Cox 1994).

A widely applicable soil test should include information about several important mechanisms of P supply and uptake. Transport of P to the root surface is controlled by the gradient of P concentration that exists between bulk soil solution and the solution at the root surface. This gradient is generated and maintained by an integration of solute transport processes and plant demand. The theory of P supply and uptake can be used to predict the concentration of P that develops at the root surface (P_{root}) in soil, and predict P uptake (Nye and Tinker 1977, Barber 1984, Smethurst and Comerford 1993a). Using such information it may be possible to predict whether P supply to a crop will be adequate under any given set of conditions, without the need for extensive empirical calibration.

The Smethurst and Comerford (1993a) model offered more flexibility than the widely used Barber and Cushman (1981) model, because it (a) accounted for variable buffer powers with changes in the concentration of solute in solution, and (b) allowed variable root-length density during the course of a simulation. The objective of this study was to examine the potential to predict P deficiency in new eucalypt plantations using a process-based approach.

Two models were used for this series of simulations, these being a Michaelis-Menten model,

the modified Smethurst-Comerford model. The Michaelis-Menten model calculated maximum P uptake from the measured root growth and P uptake kinetic parameters, assuming infinite soil P buffering ability (ie. that the concentration at the root surface was equivalent to that in bulk soil). Soil P supply was then accounted for by using the model of Smethurst and Comerford (COMP82, Mendham *et al.* 1997), which calculated the concentration of P that develops at an average root surface during the course of the experiment. The accuracy of the two methods for predicting concentration of P at the root surface in pot experiments with *Eucalyptus nitens* was compared with that of the Barber-Cushman model.

The hypothesis that a mechanistic model of P supply and uptake would be useful for predicting P deficiency in *E. nitens* on a range of soil types was tested with the glasshouse experiments described in Chapter 6.

8.2 Materials and Methods

8.2.1 Maximum predicted P uptake.

Maximum P uptake in the highest treatment of the first glasshouse experiment (P_5) was estimated from measured root growth and uptake characteristics of the roots. The uptake kinetic parameters (I_{max}, K_m, C_{min}) of the high affinity transport system of *E. nitens* roots were used (Table 8.1), because the concentration range of the experiment (0.1 - 0.6 μM) was within the range influenced by the high affinity transport system (0 - 1 μM, Figure 4.3). Roots were assumed to grow exponentially (in the form $y = a.b^x + c$, where y is root length density (cm/cm^3), x is time (d), and a, b and c were fitted parameters). The value of b determines the curvature of the relationship, and was taken from the form of shoot growth over time in the second pot experiment. The parameters a and c were fitted to give measured initial and final root lengths. The values of a, b and c are shown in Table 8.1. For the purposes of predicting maximum uptake, it was assumed that the soil was infinitely buffered,

i.e. that the concentration at the root surface was the same as the concentration in bulk soil solution. Uptake was predicted on a daily basis and summed for the 64-day period of the experiment.

Table 8.1 - Plant uptake kinetics and root growth parameters used for prediction of uptake.

Parameter	Symbol	Value
Maximal influx to the root (μmol/cm^2/sec)	I_{max}	1.98 x 10^{-7}
Michaelis constant (μmol/cm^3)	K_m	0.000374
Minimum concentration for uptake (μmol/cm^3)	C_{min}	0
Root growth *a*	*a*	0.00942
Root growth *b*	*b*	1.05
Root growth *c*	*c*	-0.00941

8.2.2 Modeling uptake by *E. nitens* in the first glasshouse experiment.

The Smethurst and Comerford (1993a) nutrient uptake model that was modified (in Mendham *et al.* 1997) to account for a lower tolerance between C_{root} and C_{min} (COMP82, referred to hereafter as the Smethurst-Comerford model) was used to predict the concentration of P at an average root surface for this series of simulations.

The quantity of soil occupied by root depletion zones was less than 78% of the pot volume (Table 6.14) in all soils, so there would have been little competition for P between the plants

during the course of the glasshouse experiments. Therefore, uptake by single plants was modeled at each treatment level. The equilibrium P buffer capacities of soils in the pot experiments are shown in Table 8.2.

Table 8.2 - Buffer capacity of soils in the two glasshouse experiments used to test the hypothesis that a process-based model could be used as an indicator of P deficiency.

Experiment	Soil	Equilibrium buffer capacity (mL/g)
Glasshouse expt. 1	Clay loam 2	23 404
Glasshouse expt. 2	Sand	575
	Silty clay loam	509
	Clay loam 1	1520

Soil factors maintained at a constant level were: soil volume, water content, bulk density, and Freundlich coefficients of P sorption (Table 8.3). The Freundlich coefficients refer to the Freundlich equation: $y = a.x^{1/b}$, where y = P sorbed onto the solid phase (µg/g), x = concentration of nutrient in solution (µg/mL), and a and b are fitted parameters. The diffusion coefficient of P in water (D_l) was described by Edwards and Huffman (1959), and the rate of water influx to the root (v) was typical of values published by Barber (1984). Water influx had little effect on predicted P uptake (see Results). The initial time step of the Smethurst-Comerford model was 0.01 seconds.

Table 8.3 - Values maintained at a constant level for every simulation of P uptake in the first pot experiment.

Parameter	Symbol	Value
Soil volume (cm^3)		1350
Water content (cm^3/cm^3 soil)	θ_v	0.4199
Bulk Density (g/cm^3)	ρ	0.8
Freundlich '*a*' coefficient	*a*	7385
Freundlich '*b*' coefficient	*b*	1.39
Maximal influx to the root ($\mu mol/cm^2/s$)	I_{max}	4.95×10^{-7}
Diffusion coefficient in liquid (cm^2/sec)	D_l	8.9×10^{-6}
Water influx to the root ($cm^3/cm^2/sec$)	*v*	5.00×10^{-7}

The measured concentration of P in solution (C_l), root radius (R_0) and root growth were different for each treatment (Table 8.4). The curvature (*b*) coefficient of root growth was estimated from growth of shoots over time, and the '*a*' and '*c*' coefficients were calculated to give the observed root length at the beginning and end of the experiment.

Table 8.4 - Values of parameters that were varied with each simulation.

Treatment	Initial conc. of P in solution[A]	Root radius	Root growth parameters[B]		
	(C_l, μM)	(R_0, cm)	*a*	*b*	*c*
P_0	0.074	0.0106	0.00254	1.05	-0.00253
P_1	0.089	0.0092	0.00492	1.05	-0.00491
P_2	0.198	0.0094	0.00931	1.05	-0.00931
P_3	0.404	0.0091	0.00874	1.05	-0.00873
P_4	0.550	0.0098	0.00851	1.05	-0.00851
P_5	0.617	0.0092	0.00945	1.05	-0.00944

[A] $CaCl_2$ P concentrations were corrected to represent solution P concentration.

[B] Equation for root growth: $y = a.b^x + c$, where y = root length density (cm/cm^3), and x = time (days), and a, b and c are fitted parameters.

An error analysis of the inputs to the Smethurst-Comerford model was conducted to examine the effect of independently changing the following parameters on uptake: concentration of P in solution (C_l), diffusion coefficient of P in water (D_l), soil water content (θ_v), water flux to the root (v), soil P buffer power (b), kinetic parameters of uptake (I_{max}, K_m) and root growth form (rg form) and rate (rg rate). Phosphorus uptake in the P_5 treatment was modeled in the error analysis, to minimize the potential influence of mycorrhizae. Mycorrhizal infection was not measured during the experiment, but it was assumed to have been minimal for two reasons: (a) the effects of possible infection would have been minimized in the highest P treatment, as has been found for soybeans (Plenchette and Morel 1996) and eucalypts (Bougher *et al.* 1990, Barrow 1977), and (b) the duration of the experiment was short

compared with the time required for mycorrhizal establishment. For example, Heinrich and Patrick (1986) observed low numbers of mycorrhizae on *Eucalyptus pilularis* seedling roots in a pot experiment at 30 days, but the main effect of ectomycorrhizal inoculation on P uptake and plant growth occurred after 96 days

Each parameter in the error analysis (i.e. C_l, D_l, θ_v, v, I_{max}, K_m, b, rg form, and rg rate) was modified individually, while the other parameters were maintained at the values shown above (Table 8.1, Table 8.3 and Table 8.4). The parameters were changed within the limits in Table 8.5. Solution P concentration was modified by a factor of 10 because rhizosphere acidification caused an increase of this magnitude in the rhizosphere around *Brassica napus* roots (Hedley *et al.* 1982a).

The range of the diffusion coefficient between 5°C and 35°C was calculated using Equation 8.1 (the Stokes-Einstein equation, from Barber 1984).

$$D_l = \frac{k_B T}{6\pi r_i \eta}, \qquad \text{Equation 8.1}$$

where k_B is the Boltzmann constant (1.38 x 10^{-16} g.cm^2/s), T is absolute temperature (K), r_i is ionic radius (cm), and η is water viscosity.

The uptake kinetic parameters, I_{max} and K_m were modified 5-fold and 2-fold, respectively, which was the approximate range observed by Jungk *et al.* (1990) for *Zea mays* and *Glycine max*, and by Cogliatti and Santa-Maria (1990) for *Triticum aestivum*, with different pre-treatment concentrations (Table 2.5).

Buffer power was changed by an order of magnitude above and below that used in the standard simulation, because short-term adsorption isotherms may significantly over- or under-estimate the P buffer power of the soil, depending on the influences of hysteresis and

long-term sorption (Barrow 1987, P. W. Moody, pers. comm.). Water velocity to the root was changed by 2 orders of magnitude above and below that used in the standard simulation, due to uncertainty over the actual water velocity to the root. Soil water content range was modified within the limits that were maintained in pots during growth.

Root growth was independently varied in two ways. The form of root growth in the error analysis ('b' parameter) was changed from linear ($b = 1$) to exponential ($b > 1$), and the length of roots ('a' parameter) was modified ±50% of that measured, to account for errors in estimation of root growth rate (Section 3.6.3). For each 'a' or 'b' parameter that was modified in the error analysis, the other parameters of root growth were recalculated to obtain the known initial and final root lengths.

For simulations with different buffer powers, the Freundlich 'a' coefficient was set to the value of the buffer power, and the Freundlich 'b' coefficient was set to 1. This gave constant buffer powers during each simulation. Buffer power changed by approximately 1% in the standard simulations, so this simplification was justified.

Table 8.5 - Minimum and maximum values of parameters in the error analysis of the P_5 treatment in glasshouse experiment 1.

Variable	Original value	Modified values	
		Minimum	Maximum
Concentration in solution (C_l, μM)	0.617	0.0617	6.17
Diffusion coefficient in liquid (D_l, $cm^2/sec.$)	8.90×10^{-6}	4.87×10^{-6}	1.14×10^{-5}
Water content (θ_v, cm^3/cm^3)	0.420	0.370	0.470
Velocity of water to the root surface (v, $cm^3/cm^2/sec.$)	5×10^{-7}	5×10^{-9}	5×10^{-5}
I_{max} (μmol/cm^2/sec.)	1.98×10^{-7}	3.96×10^{-8}	9.90×10^{-7}
K_m (μM)	0.362	0.181	0.724
Buffer capacity (*b*, Freundlich '*a*' coefficient)	1.73×10^4	1.73×10^3	1.73×10^5
Root growth rate ('*a*' parameter)	0.00942	0.00471	0.0141
Root growth form ('*b*' parameter)	1.05	1	1.10

The Barber-Cushman method of calculating the concentration at the root surface used an analytical solution, which is more accurate under certain conditions than the numerical solution used by the Smethurst-Comerford model (see Chapter 2). To validate that the numerical solution used in the Smethurst-Comerford model was giving accurate results in the conditions of the glasshouse experiments with *E. nitens*, the concentration of P at the root surface predicted by the Smethurst-Comerford model was compared with that predicted by the Oates and Barber (1987) model over the duration of the experiment. Constant values used

in the Barber-Cushman model were water velocity to the root (v, Table 8.3), and uptake kinetic parameters (I_{max}, K_m and C_{min}, Table 8.1). Variables changed in each simulation were the concentration of P in solution (C_l), root radius (R_0), diffusion coefficient (D_e), buffer capacity (b), and root half-distance (R_1), see Table 8.6. The Smethurst-Comerford model calculated D_e, b and R_1 parameters during the course of each simulation, whereas the Barber-Cushman model assumed that they were maintained at the initial value. During the course of the Smethurst-Comerford model simulations, D_e and b changed by no more than 6%, while the value of R_1 had no influence on predicted concentration of P at the root surface because depletion zones did not overlap.

Table 8.6 - Variables changed for modeling uptake in each treatment in the Barber-Cushman model.

Simulation	D_e (cm^2/s)	Buffer Capacity	R_1 (cm)
P_0	1.03×10^{-10}	23 405	2.40
P_1	1.09×10^{-10}	22 245	1.73
P_2	1.37×10^{-10}	17 740	1.25
P_3	1.67×10^{-10}	14 529	1.30
P_4	1.82×10^{-10}	13 323	1.31
P_5	1.88×10^{-10}	12 901	1.25

A spreadsheet was used to predict uptake from the root surface concentrations predicted by the Smethurst-Comerford model and Barber-Cushman model. The spreadsheet allowed a

common root growth model (Table 8.4) to be used. Total time, *t*, of the simulation was divided into 0.2 day time steps. Uptake at each time step was calculated from the average concentration predicted at the root surface and the quantity of roots calculated by the root growth model. Total uptake was predicted by summing uptake calculated at each time-step. There was good agreement (± 10%) between uptake predicted by this method and that predicted by the Smethurst-Comerford model.

8.2.3 Modeling uptake by *E. nitens* in the second glasshouse experiment.

Initial time step, soil volume, diffusion coefficient of P in water, water flux to the root, and kinetics parameters were the same as in the first experiment (Table 8.1 and Table 8.3). Variables maintained at a constant level within each soil type were buffer power (Freundlich parameters), water content, and bulk density (Table 8.7).

Table 8.7 - Soil-type specific parameters used in the Smethurst-Comerford model for predicting P uptake in the second glasshouse experiment.

Soil	Water content	Bulk Density	Freundlich parameters	
	(cm^3/cm^3)	(g/cm^3)	*a*	*n*
Silty clay loam	0.462	0.8	20.452	5.282
Clay loam	0.5339	0.7	1570.2	1.062
Sand	0.0449	1.4	13.074	3.746

Parameters that were varied in each treatment were the concentration of P in soil solution, root radius and root growth parameters (Table 8.8). The curvature coefficient of root growth (*b*) was calculated from shoot dry weight over time, and the '*a*' and '*c*' coefficients of root

growth were fitted to the measured root length at the beginning and end of the experiment.

Table 8.8 - Values of the variables that were different for each treatment, and used for prediction of uptake in the Smethurst-Comerford model in the second glasshouse experiment.

Soil	Treatment	Concentration of P in solution[A] (μM)	Root radius (mm)	Root growth parameters[B]		
				a	*b*	*c*
Silty clay loam	P_0	0.21	0.14	0.01155	1.027	0.00244
	P_1	0.29	0.23	0.00083	1.072	0.00362
	P_2	0.34	0.21	0.00056	1.078	0.00389
	P_3	0.38	0.21	0.00195	1.064	0.00358
Clay loam	P_0	0.27	0.11	5.9×10^{-6}	1.127	-0.0237
	P_1	0.28	0.10	0.00938	1.05	-0.0141
	P_2	0.38	0.12	4.9×10^{-5}	1.127	0.00440
	P_3	0.49	0.10	0.00052	1.097	4.39×10^{-3}
Sand	P_0	0.18	0.13	0.0283	1.014	-0.0348
	P_1	0.34	0.12	0.03129	1.024	-0.0311
	P_2	0.88	0.17	0.00066	1.08	0.00379
	P_3	1.05	0.14	0.00069	1.08	0.00376

[A] $CaCl_2$ P concentrations were corrected to represent solution P concentration.

[B] Equation for root growth: $y = a.b^x + c$, where y = root length density (cm/cm^3), and x = time (days), and a, b and c are fitted parameters.

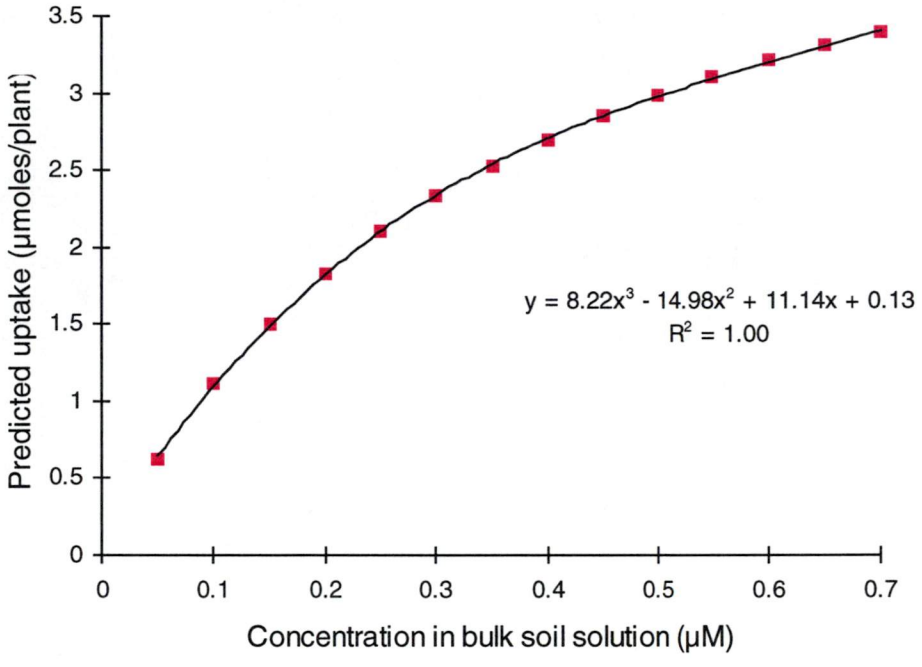
8.3 Results

8.3.1 Maximum predicted P uptake.

Maximum predicted P uptake in the P_5 treatment (using the measured Michaelis-Menten

uptake parameters and root growth) was 3.24 µmoles at a concentration of 0.62 µM P in soil solution (Figure 8.1), whereas observed uptake in the experiment was 5.27 µmoles. Hence root surface area or uptake parameters were not described adequately.

Figure 8.1 - Relationship between external concentration and predicted uptake.



Errors in measured root length were ±20% of the estimated value (Section 3.6.3) and the form of root growth was not measured, so root length in the model was increased by 50% to account for those errors. The kinetic parameters were measured on roots that had been pretreated at 10 µM P, whereas the roots in this experiment were subject to concentrations less than 0.6 µM P, and the value of I_{max} changes with pretreatment concentration (e.g. Jungk *et al.* 1990, Dunlop *et al.* 1997). Jungk *et al.* (1990) observed a 5-fold increase in I_{max} as the pretreatment concentration decreased from 10 µM to 0.1 µM, so I_{max} was increased 5-fold to investigate the effect of changing I_{max} on predicted uptake.

Increasing root length by 50% increased the predicted uptake to 4.87 µmoles, and increasing

I_{max} 5-fold increased predicted uptake to 16.23 μmoles (Table 8.9). Maximum predicted uptake (24.34 μmoles) occurred when both factors were increased. The value of I_{max} had the main influence on predicted uptake, and underestimation of that parameter may have been the reason for the underprediction of uptake.

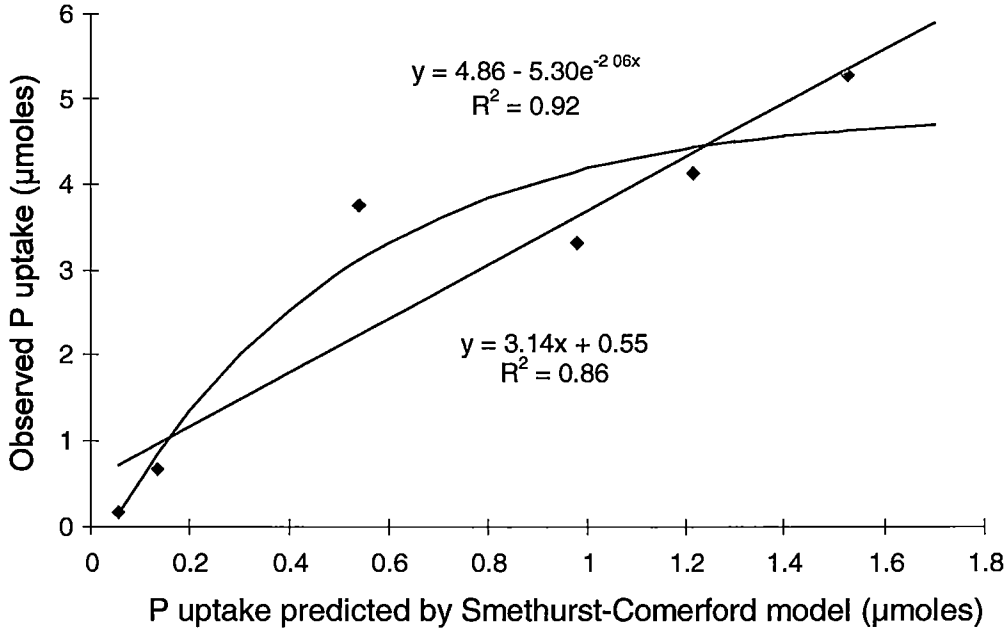
Table 8.9 - Effect of changing I_{max} and root length parameters on predicted uptake at 0.62 μM P in solution at the root surface.

	Measured I_{max}	5-fold I_{max}
Measured root length	3.24	16.23
150% of measured root length	4.87	24.34

8.3.2 Modeling uptake by *E. nitens* in the first glasshouse experiment.

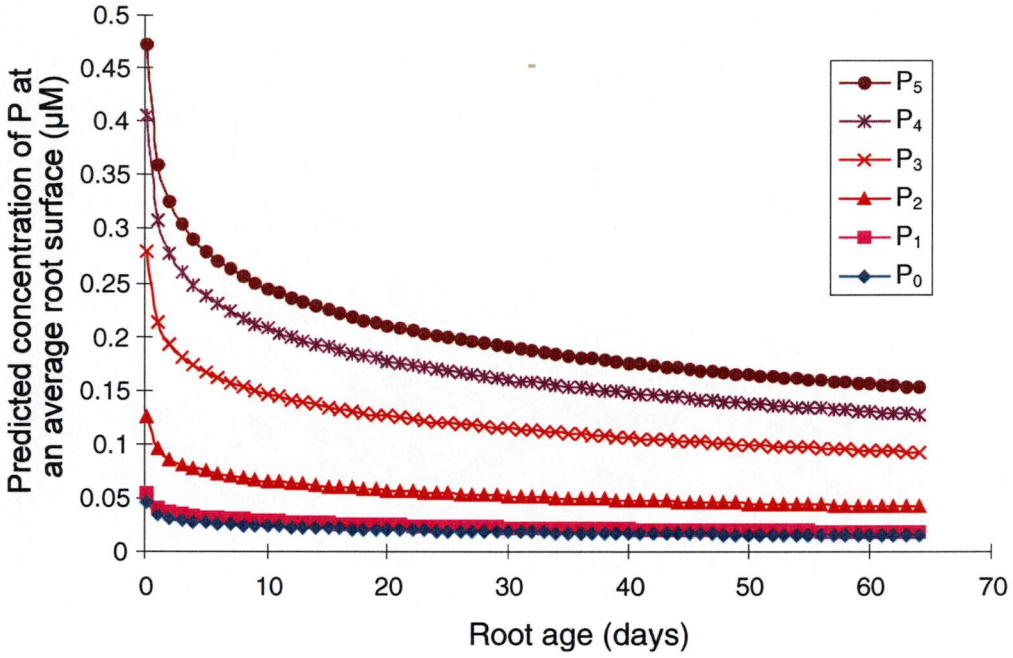
Uptake predicted by the Smethurst-Comerford model was approximately one-third of that observed in the experiment (Figure 8.2). The relationship between observed and predicted P uptake was well described by a Mitscherlich model ($y = 4.86 - 5.30e^{-2.06x}$, $R^2 = 0.92$), and all treatments except P_2 were well aligned with a linear regression ($y = 3.14x + 0.55$, $R^2 = 0.86$). Even though it was described by only one point, the Mitscherlich model was favoured because it had a higher R^2 coefficient, and based on an ANOVA in Chapter 6, which showed that uptake in the four highest treatments was not significantly different.

Figure 8.2 - Relationship between observed P uptake, and that predicted by the Smethurst-Comerford model in the first glasshouse experiment.



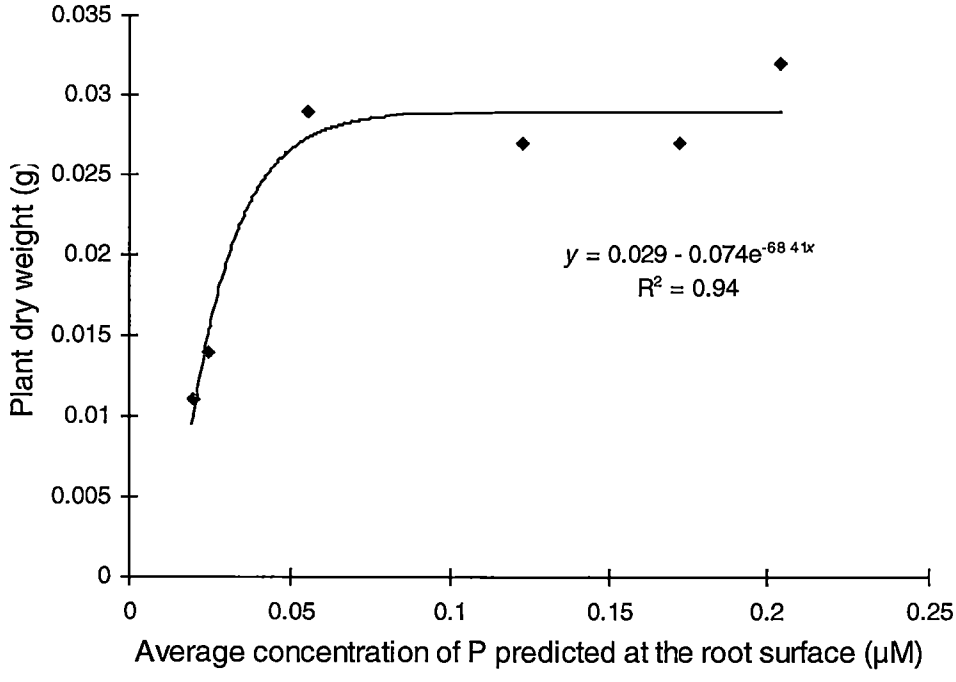
The predicted concentration of P in solution at the root surface decreased during the course of the experiment, with 82% of the decrease occurring up to 20 days into the experiment (Figure 8.3). The average predicted root surface concentrations of P over the length of the experiment were calculated and used for further comparisons with plant dry weight and P uptake.

Figure 8.3 - Predicted concentration of P at an average root surface in each treatment, during the course of the experiment.



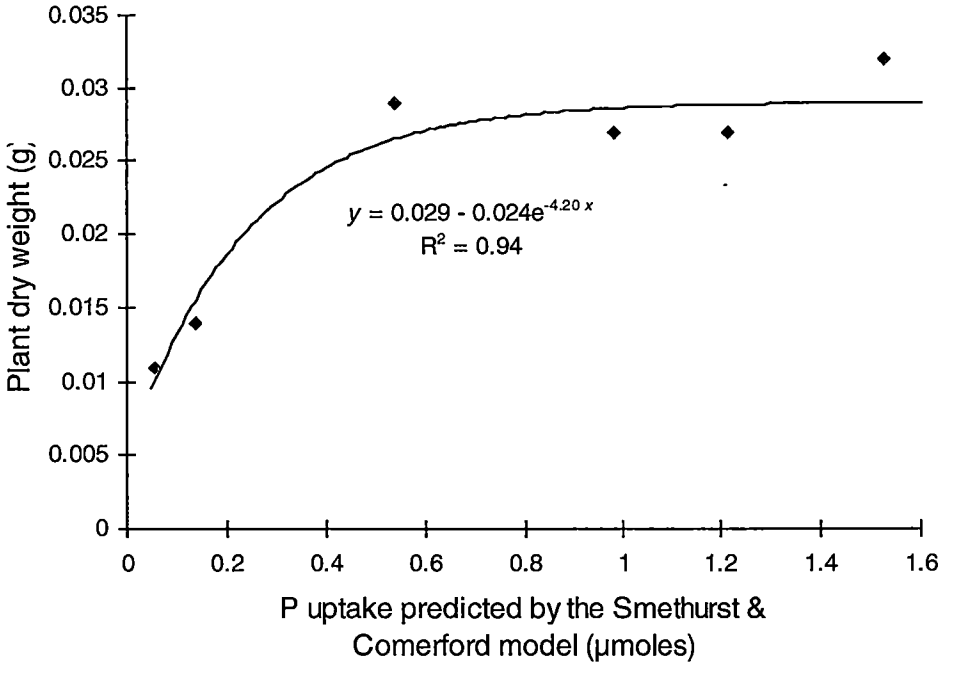
The relationship between plant dry weight and average predicted concentration of P at the root surface was asymptotic (Figure 8.4), and well described by a Mitscherlich model. The equation for the Mitscherlich curve was: $y = 0.029\text{-}0.074e^{-68.41x}$ ($R^2 = 0.94$). Ninety percent of maximum growth occurred at a predicted concentration of P at the root surface of 0.0485 µM.

Figure 8.4 - Relationship between plant dry weight and average predicted concentration at the root surface.



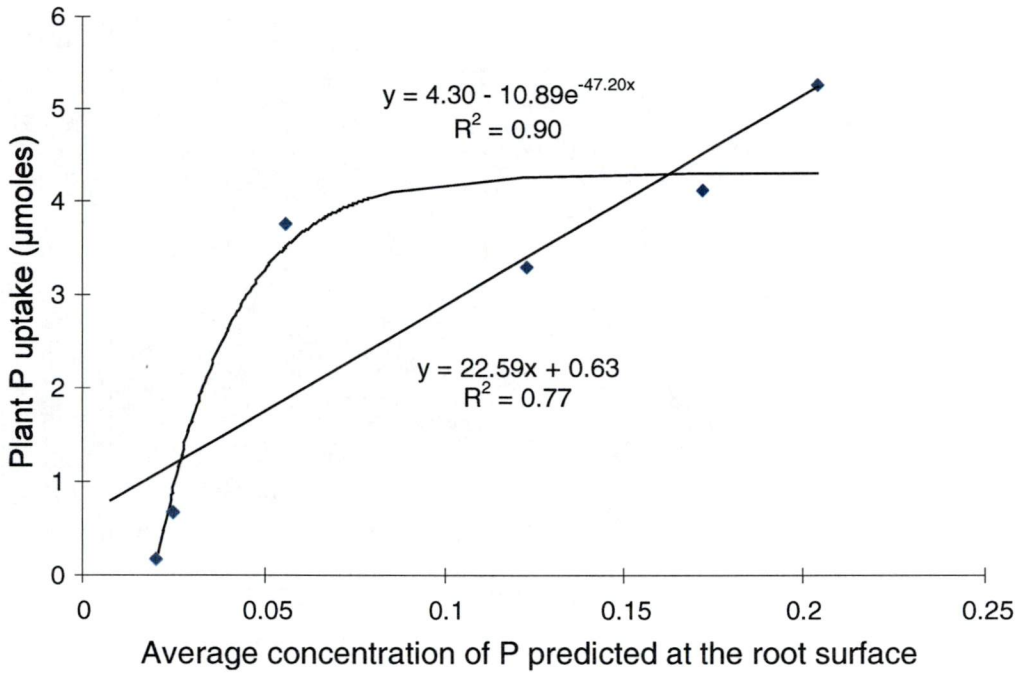
The relationship between uptake predicted by the Smethurst-Comerford model and plant dry weight was also asymptotic (Figure 8.5), with 90% of maximum growth occurring at a predicted uptake of 0.50 µmoles.

Figure 8.5 - Relationship between plant dry weight and P uptake predicted by the Smethurst-Comerford model.



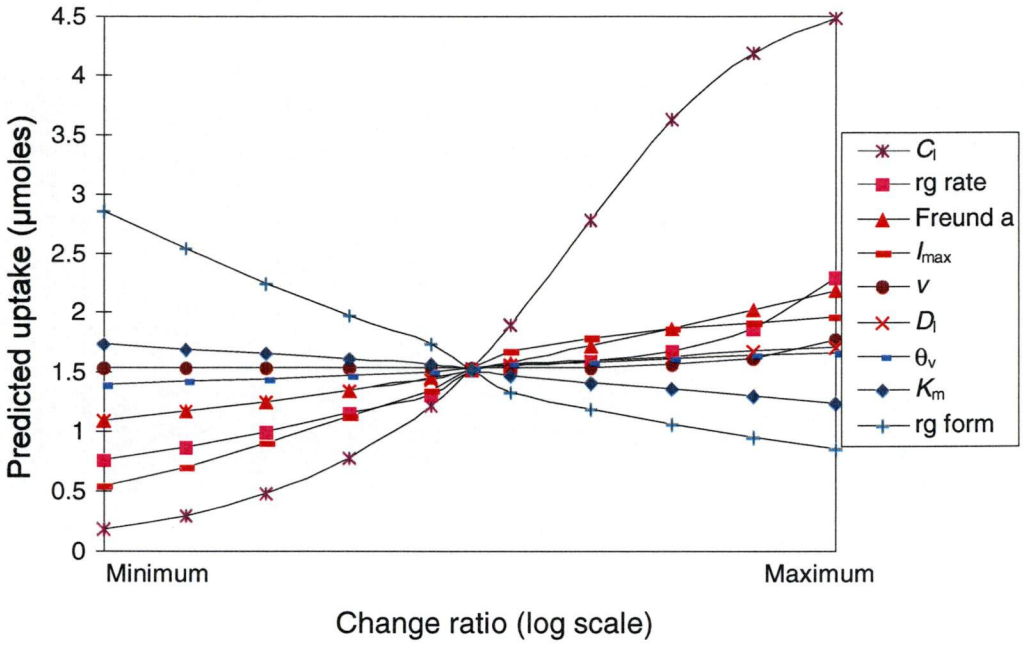
The relationship between plant P uptake and average predicted concentration at the root surface (Figure 8.6) was described well by a Mitscherlich model ($y = 4.30 - 10.89e^{-46\,20x}$, $R^2 = 0.90$), and a linear model ($y = 21.9x + 0.63$) was also well correlated ($R^2 = 0.77$).

Figure 8.6 - Relationship between observed P uptake and average predicted concentration of P at the root surface.



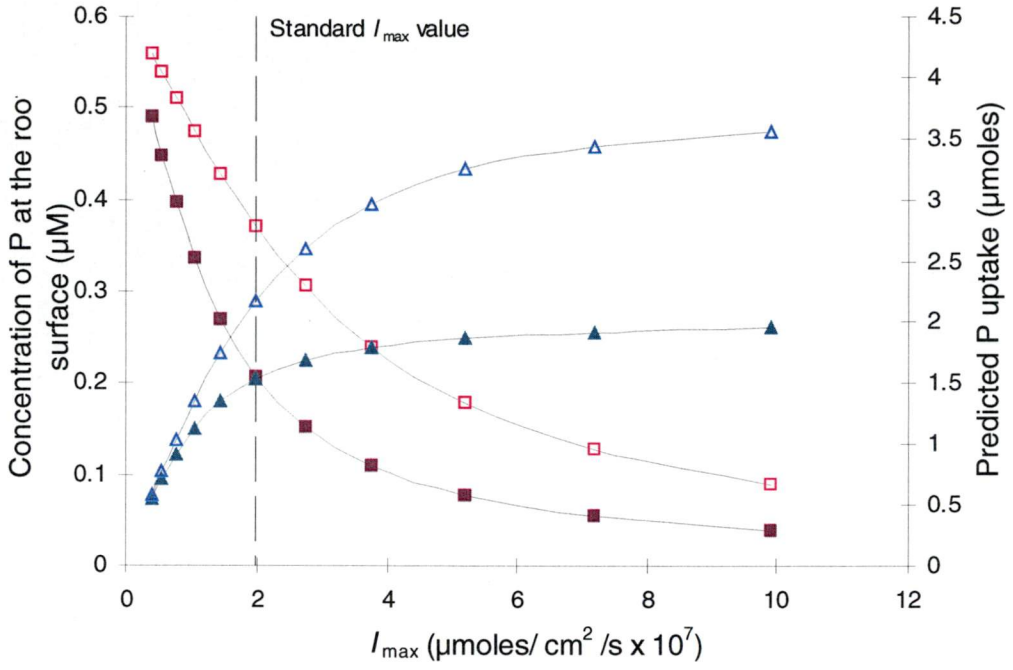
At the maximum value of each of the parameters in the error analysis, uptake was ranked (from lowest to highest): exponential root growth form < K_m < $\theta_v \approx D_l \approx v$ < I_{max} < b < rg rate < C_l, (Figure 8.7). A 10-fold increase in the concentration of P in the external solution had the greatest effect on predicted uptake (4.49 µmoles), while the next two parameters (ie. root growth rate and buffer power) gave a predicted uptake of approximately 2.8 µmoles at their maximum level in the error analysis. High K_m reduced predicted uptake, as did the root growth form with the highest curvature.

Figure 8.7 - Effect of changing some parameters of the Smethurst-Comerford model within the maximum and minimum limits in Table 8.5.



Increasing the I_{max} parameter in the Smethurst-Comerford model (Figure 8.7) had a lesser effect on predicted uptake than when infinite buffering was assumed (Table 8.9), because increased I_{max} resulted in reduced concentration of P at the root surface (Figure 8.8). Increased soil buffer power in the model led to greater concentrations of P at the root surface, and hence greater predicted uptake by the plants.

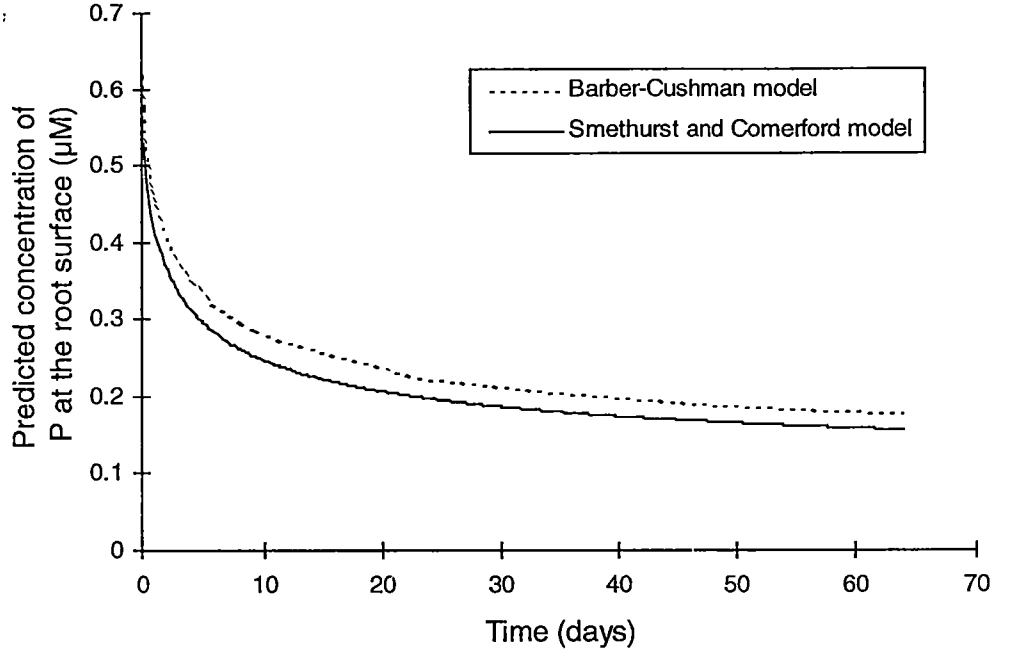
Figure 8.8 - Effect of changing I_{max} on the predicted concentration of P at the root surface and uptake (Δ = Predicted P uptake, ■ = Concentration at the root surface, solid symbols = measured buffer power, hollow symbols = 10 times normal buffer power).



8.3.2.1 Comparison of uptake predictions between the Smethurst-Comerford and Barber-Cushman models.

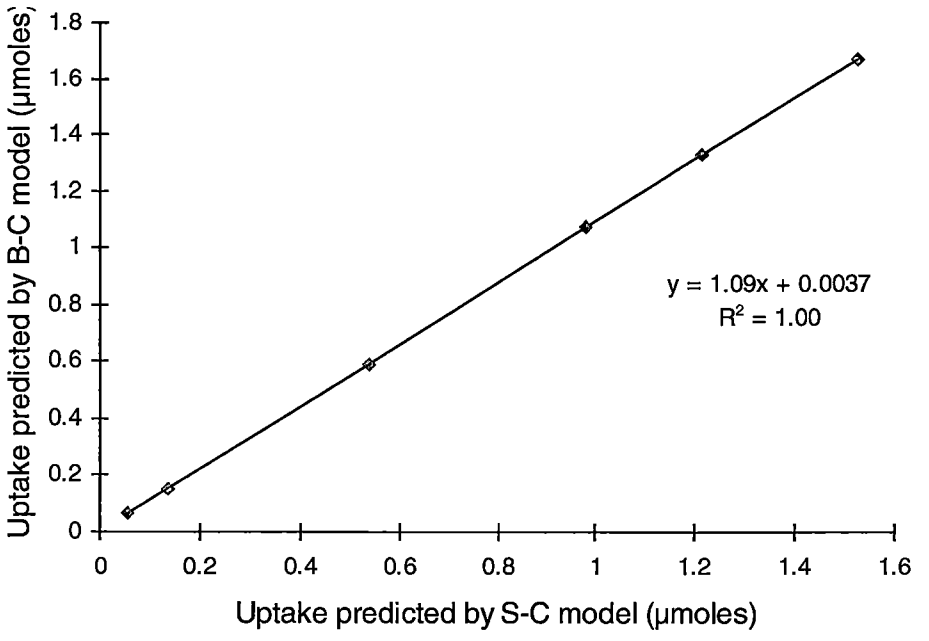
The P concentration predicted at the root surface by the Barber-Cushman model followed a similar trend over time but was between 13 and 21 percent higher than that predicted by the Smethurst-Comerford model (Figure 8.9).

Figure 8.9 - Root surface concentration of P predicted by the Barber-Cushman model and Smethurst-Comerford model.



Uptake predicted by the Barber-Cushman model and Smethurst-Comerford model was highly correlated ($R^2 = 1.00$), and related by a linear trend (Figure 8.10, $y = 1.09x + 0.0037$), but the slope of the regression indicated that the Barber-Cushman model predicted 9% more uptake than the Smethurst-Comerford model.

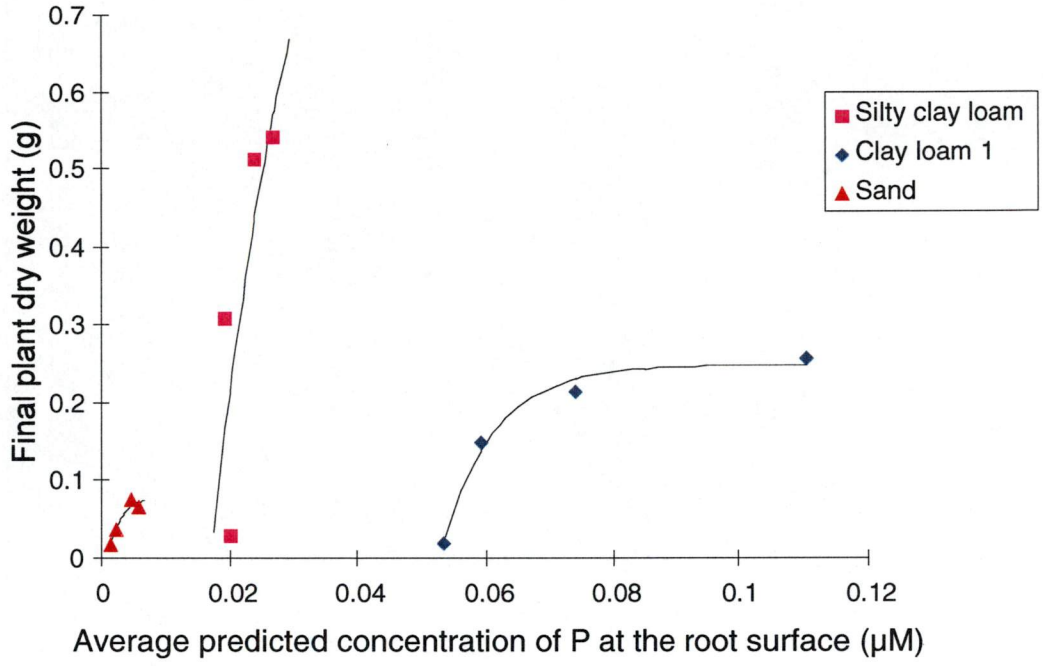
Figure 8.10 - Relationship between uptake predicted by the Barber-Cushman and Smethurst-Comerford models.



8.3.3 Modeling uptake by *E. nitens* in the second glasshouse experiment.

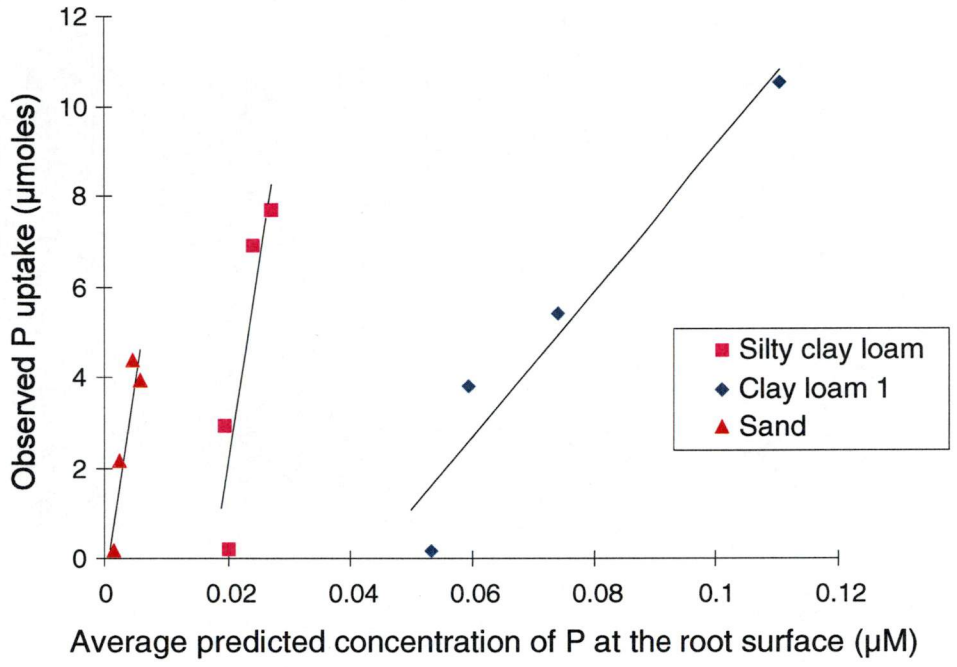
The relationship between shoot dry weight and concentration at the root surface was different for each soil type (Figure 8.11). Mitscherlich equations describing the relationship between plant dry weight and the concentration of P at the root surface were: $y = 1.20 - 3.67e^{-65.55x}$ ($R^2 = 0.63$) for the silty clay loam, $y = 0.25 - 160e^{-123x}$ ($R^2 = 0.99$) for the clay loam, and $y = 0.079 - 0.13e^{-509x}$ ($R^2 = 0.92$) for the sand. The relationships between the shoot dry weight and predicted uptake was also soil type specific (data not shown).

Figure 8.11 - Relationship between the final shoot dry weight and average predicted concentration at the root surface.



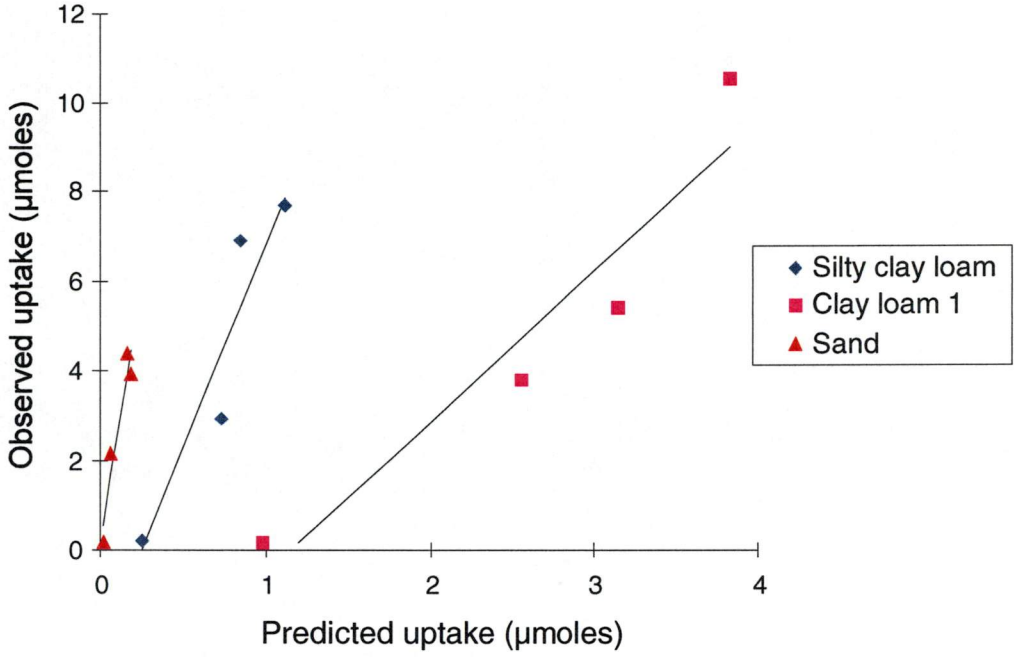
The relationship between uptake and the concentration of P at the root surface was soil-type specific (Figure 8.12), but within each soil, a near-linear relationship was observed. Equations for the relationships were: $y = 881x - 15.4$ ($R^2 = 0.79$) for the silty clay loam, $y = 161x - 7.01$ ($R^2 = 0.93$) for the clay loam, and $y = 877x - 0.50$ ($R^2 = 0.84$) for the sand.

Figure 8.12 - Relationship between uptake and predicted concentration of P at the root surface in 3 soil types.



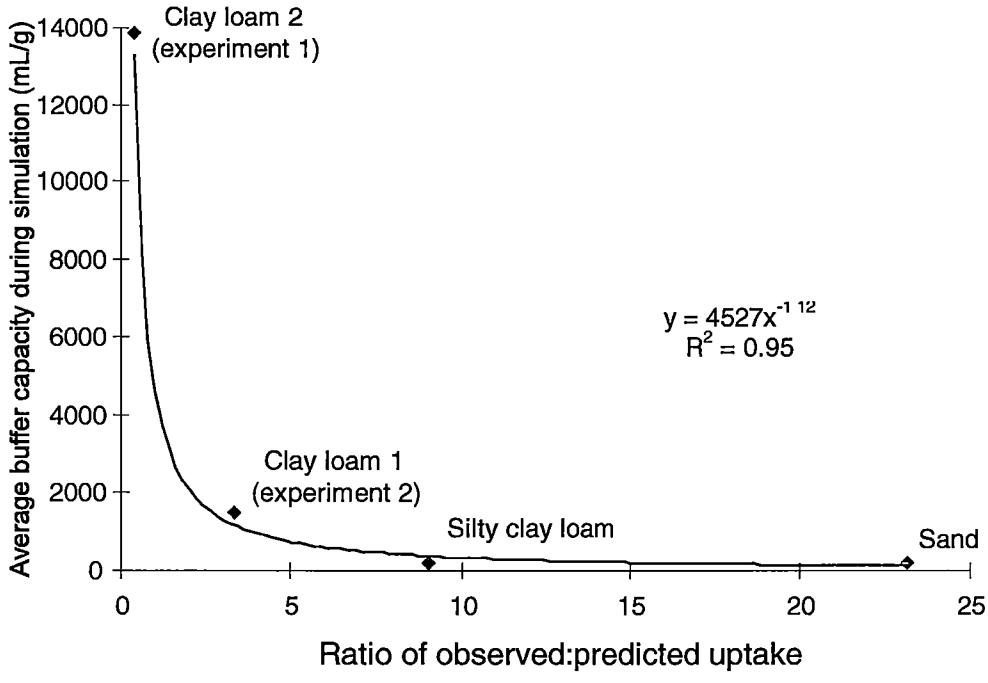
Phosphorus uptake predicted by the Smethurst-Comerford model was lower than that observed in each treatment (Figure 8.13). Observed uptake was about 9-fold that predicted in the silty clay loam (equation: $y = 9.03x - 2.25$, $R^2 = 0.88$), 3-fold that predicted in the clay loam ($y = 3.36x - 3.88$, $R^2 = 0.90$), and 23-fold that predicted in the sand ($y = 23.14x + 0.046$, $R^2 = 0.92$).

Figure 8.13 - Relationship between observed and predicted uptake in the second glasshouse experiment.



The accuracy of prediction of uptake was related to soil P buffer power (Figure 8.14). Predicted uptake approximated observed uptake in soils of high P buffer power, but the ratio of observed to predicted uptake decreased as buffer power decreased.

Figure 8.14 - Relationship between buffer power and ratio of predicted to observed uptake in the two glasshouse experiments.



8.4 Discussion

Uptake was under-predicted by the measured root length and uptake kinetic parameters, even when infinite soil P buffering was assumed (Figure 8.1). The reason for under-prediction was probably poor estimation of either root surface area or uptake kinetic parameters. Root surface area was estimated using Method 3.6.3, and errors associated with that method were approximately ±20%, but root hairs and mycorrhizal infection were not accounted for. Depletion zones calculated by the Smethurst-Comerford model were approximately 5000 μm, while the root hairs were approximately 50 μm, so root hairs were considered to have a minimal influence on P uptake. It was also assumed that mycorrhizal infection would have had minimal effect on P uptake and growth in this experiment, because (a) mycorrhizae were not found on the roots during root diameter measurements (visual observation), (b) the effects of possible mycorrhizal infection would have been minimal at higher soil P, because

infection increases growth only in soils low in available P (Bougher *et al.* 1990), and (c), the time of the experiments was less than that required for effective mycorrhizal contributions in the experiment of Heinrich and Patrick (1986).

The value of I_{max} was a likely cause of under-prediction, because plants in the uptake kinetics experiment were grown in a 10 μM P solution, without the normal complement of rhizosphere microorganisms. High P pretreatment concentrations result in lower I_{max} values (e.g. Jungk *et al.* 1990, Cogliatti and Santa Maria 1990, Dunlop *et al.* 1997), and inoculation of roots with soil microorganisms increased I_{max} of cabbage roots (Temple-Smith and Menary 1974), and *Pinus radiata* roots (Bowen 1969). Concentrations of P in soil solution at the roots of plants in this experiment were less than 0.6 μM. Five-fold increases in I_{max} were observed by Jungk *et al.* (1990) for two species over a similar pretreatment concentration range, and an increase in I_{max} of this magnitude increased predicted P uptake from 3.24 μmoles to 16.23 μmoles in the Michaelis-Menten only model (Table 8.9). Observed P uptake (5.27 μmoles) was well within this range, so errors in I_{max} easily accounted for under-prediction by the Michaelis-Menten model. Increasing root growth by 50% caused an increase in predicted uptake to 4.87 μmoles, which was below observed uptake.

When soil supply was considered, predicted concentration of P at the root surface was reduced to less than half of that in bulk soil solution after 11-14 days in the highly P buffered soil (Figure 8.3). Hence P uptake predicted by the Smethurst-Comerford model was a small proportion of observed uptake. Both the Smethurst-Comerford and Barber-Cushman models predicted similar root surface concentrations (Figure 8.9 and Figure 8.10), indicating that the analytical solution of the Smethurst-Comerford model was as precise as the numerical solution of the Barber-Cushman model.

Increasing I_{max} 5 times in the Smethurst-Comerford model did not increase uptake by the same magnitude that it did when buffer power was assumed to be infinite (Table 8.9), because it was accompanied by a resultant decrease in the predicted concentration of P at the

root surface (Figure 8.8).

Silberbush and Barber (1984) also found that the Barber-Cushman model under-predicted P uptake by field-grown soybeans in conditions of low P availability. At high native concentrations of P in the soil solution, model predictions were similar to observed uptake, but at low soil P, the model under-predicted uptake by 70%. They postulated that increased uptake at low P concentrations was due to acidification of the rhizosphere, and/or the presence of mycorrhizae. Mycorrhizae may have been the cause of the higher-than-predicted uptake in the current experiment, but a combination of higher buffer power and higher I_{max} was more likely. When buffer power was increased 10-fold in the simulations with increased I_{max} (Figure 8.8), predicted uptake increased to 4.2 μmoles, which was close to that observed (5.27 μmoles). Buffer power may be underestimated by short-term adsorption curves (Barrow, 1983b), and rhizosphere processes such as acidification and release of phosphatases may increase the effective buffer power experienced by roots. Rhizosphere acidification (e.g. Gillespie and Pope 1990a and 1990b, Grinsted *et al.* 1982, Saleque and Kirk 1995), and/or phosphatase release (eg. Hedley *et al.* 1982b, McKenzie *et al.* 1995) are mechanisms employed by plants to increase the concentration of P in the soil solution at the root surface (Barber 1984, Barrow 1984). Lower pH has been shown to increase competition between anions for sorption to goethite, and thus increase P availability (Geelhoed *et al.* 1997), and phosphatase enzymes release P bound in organic forms (Marschner 1995). Neither of these processes have been quantified for eucalypts, but acidification of the *E. nitens* rhizosphere was likely, as that species has demonstrated a preference for the ammonium form of nitrogen, even at low pH (Garnett 1996). Preferential uptake of cations over anions causes net excretion of H^+ ions by roots to maintain charge balance (Marschner 1995), which increases acidity around the root. Nye (1981) showed that a decrease in pH at the root surface of approximately 1 unit was theoretically possible (at an initial pH of 5.0) by accounting for rate of H^+ efflux and subsequent diffusion. The experiment modeled by Nye (1981) was similar to the situation presented here. For example, the H^+ efflux (3×10^{-12} mol/cm^2/s) used by Nye

(1981), was similar to that (5-25 x 10^{-12} mol/cm^2/s) found by Garnett (1996) for *E. nitens* roots in solution culture. Also, the soil pH buffer capacity (11 umoles/mL/unit pH) used by Nye (1981) was similar to the range of pH buffer capacities for Ferrosols (43-213 umoles/mL/unit pH) presented by Moody (1994). However, Nye (1981) did not take into account secretion of organic acids, which would also tend to reduce pH at the root surface.

The predicted concentration at the root surface for 90% of maximal growth was 0.05 μM, which was approximately 10% of the K_m value (Figure 4.3), and of the concentration of P in bulk soil solution required for optimal growth (Figure 6.24). Smart and Bloom (1993), and Clarkson (1985) hypothesised that the uptake mechanism of plant roots could provide the required amount of N if it was available at the roots at near the K_m concentration. The current study showed that optimal uptake of P occurred at near the K_m in bulk soil solution (Figure 8.1). Although optimal uptake occurred at very low predicted concentrations at the root surface (Figure 8.4, Figure 8.11), uptake was underpredicted in all cases, and the actual concentration at the root surface was probably closer to that in bulk soil solution, as demonstrated by the observation that predicted uptake was close to observed when soil supply was not taken into account (Figure 8.1).

The relationships between predicted and observed uptake (Figure 8.2, Figure 8.13) were linear in 3 of the 4 soils, but uptake in the P_2 treatment of the highly P-sorbing soil did not follow the linear trend (Figure 8.6, Figure 8.2). Although the model generally produced a linear relationship between observed and predicted uptake, the relationship was quite different for each soil type (Figure 8.2, Figure 8.13). The slope of the relationship was well correlated with buffer power (Figure 8.14), indicating that the model did not account for buffer power adequately.

The advantage of a linear relationship between predicted and observed uptake is that such a relationship would be useful for assessment of P deficiency under field conditions. Maximum potential growth of plants in the field is dependent on solar radiation, climate and soil factors,

and so the relationship between a simple index of P availability and growth may only be applicable for the same level of growth in the field. However, if expected growth rate could be predicted from net primary productivity models (such as that for *Eucalyptus globulus* by Battaglia and Sands, 1997), a linear relationship between predicted concentration at the root surface and uptake could be used to ensure that enough P was available to the plant to maximize potential growth rate.

The error analysis showed that concentration of P in soil solution was most likely to influence P uptake. Increased P release due to rhizosphere acidification or phosphatase activity would be a potential cause of increased P concentration at the root surface. The error analysis also showed that uncertainty about buffer power, I_{max} and root growth rate were also potential causes of under-prediction of P uptake. Factors that did not considerably affect predicted uptake were the velocity of water to the root surface, diffusion coefficient, and volumetric soil water content. The ranking of the importance of the various parameters in this study was different to the sensitivity analysis of Silberbush and Barber (1983), but the parameters in that study were changed by a fixed amount (ie. from half to double the standard value), which was not necessarily the range that could be expected for each parameter. For example, root growth rate in that study was found to have the most influence on predicted P uptake, followed by concentration of P in solution, and root radius. For a given situation, a doubling of root growth rate is unlikely, but the concentration of P in solution is likely to more than double, especially if processes such as acidification and phosphatase release occur in the rhizosphere.

A useful test of potential P deficiency could be developed using a modeling approach, but would require empirical calibration with soil buffer power. Although only four soils were used in the current experiments, a good trend between buffer power and predicted uptake was obtained (Figure 8.14). More soils would need to be tested, preferably over a longer time scale for this relationship to be elucidated definitively. If there was a consistent relationship

between buffer power and the ratio of uptake predicted by the model to actual uptake, a test could be developed that was relatively soil-type independent. Such a test may not require empirical calibration with a wide range of soils, merely knowledge of the buffering characteristics of each soil.

Modeling of P uptake in the field was not undertaken, as more knowledge about P supply and uptake was required for accurate prediction, even in the glasshouse experiments. Sources of error would have been even greater in the field experiments.

9. General Discussion

This study has characterised soil P available to establishing temperate eucalypt plantations. Although most of the research presented in this thesis was conducted using eucalypt seedlings less than 1 year of age, the results are applicable to older *Eucalyptus* plantations because the main response to P fertilizer occurs during the first year, and such responses are generally maintained or increased over the duration of the rotation (Schönau and Herbert 1989).

Published studies have shown that P availability soon after planting influences maximum growth later in the rotation, but later fertilization has little effect on growth (see Chapter 2). One possible reason why this is the case was presented by Miller (1989) - seedlings are almost entirely dependent on soil reserves of nutrients for early growth, but P is taken up and stored within the plant as growth proceeds. Hence, older trees rely less on soil reserves, and more on stored P. Miller (1989) suggested that this process occurs over a period of months after planting, rather than years. Another explanation why early P nutrition has an important influence later in the rotation can be drawn from horticultural crops. For example, yield of tomatoes is determined by P availability very early in development (Menary 1967), due to suppression of cytokinin production by roots under conditions of P stress, which in turn decreased flower bud initiation (Menary and Van Staden 1976). Although similar phytohormonal effects in tree species have not been studied, cytokinin influences cambial activity (Wareing and Philips, 1981). It is feasible that phytohormonal signals from seedling roots could dictate rates of growth in older trees.

Handreck (1997) suggested that Australian native plants evolved mechanisms to utilize low available soil P because this is the situation in most Australian soils (Beckman 1983). This assertion was supported by findings of the current study which showed that *E. nitens* roots had a low requirement for P in solution, compared with other species.

9.1 Plant control of P uptake

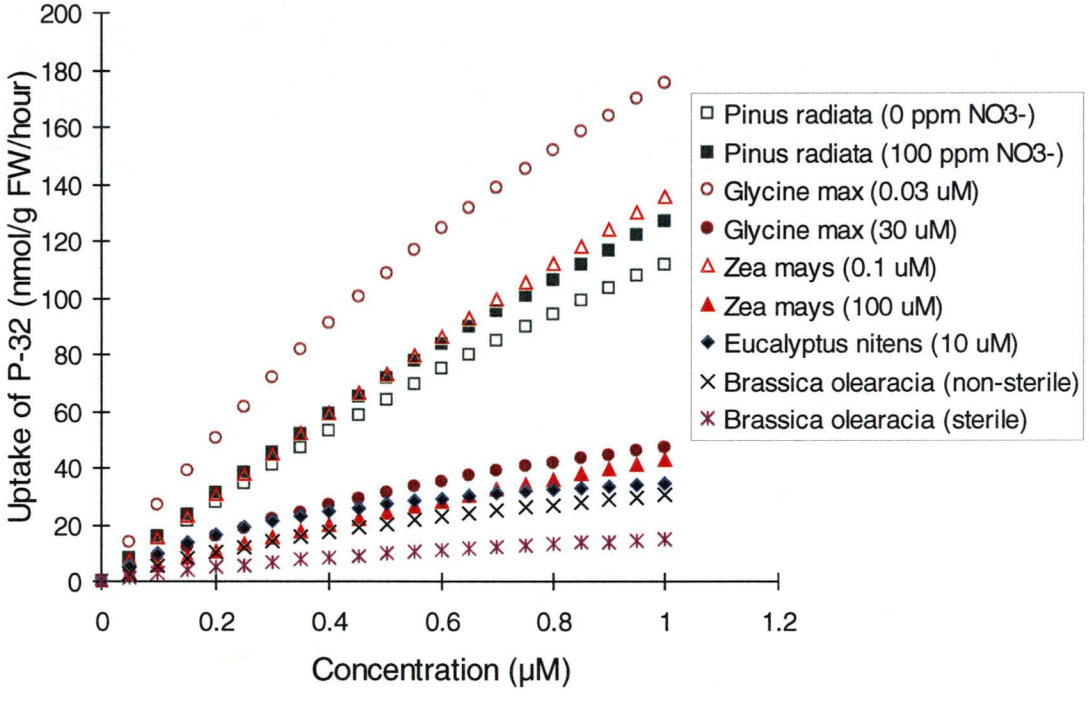
Although P uptake by roots is a complex process, the rate limiting step has been hypothesised to be a carrier in the plasma membrane with enzymic properties (Epstein and Hagen 1952). Enzyme kinetics have adequately described P uptake at low concentrations in a number of studies (Table 2.5), and it is generally accepted that high affinity uptake is mediated by an enzymic carrier. Mechanistic interpretations of observed uptake characteristics may or may not be valid, but K_m values are often used for comparison of affinity of transport mechanisms for a given nutrient. The value of K_m appears to be an intrinsic property of a given system, because it is little influenced by pretreatment concentration (Jungk *et al.* 1990) or microbial complement (Temple-Smith and Menary 1974). In contrast, the value of I_{max} of roots in soil is likely to depend on soil temperature [see reanalysis of Carter and Lathwell (1967) data, in Chapter 2] and pretreatment concentration (Lee 1982, Jungk *et al.* 1990, Dunlop *et al.* 1997). The low K_m of the high affinity transport mechanism of *E. nitens* roots indicated that *E. nitens* roots may have a low affinity for P compared with other species. The concentration range of the high affinity transport mechanism of *E. nitens* seedling roots (0 - 1 μM) was in the range of soil solution P concentrations typically present in forest soils (McLaughlin 1996, Smethurst *et al.* 1997).

Widely different combinations of kinetic parameters (I_{max} and K_m) can often describe a similar uptake curve at low concentrations, for example, some of the published kinetic parameters reviewed in Section 2.3.4 are shown graphically in Figure 9.1. Although *E. nitens* roots had a low K_m, rates of uptake were similar to that for other species over the 0 - 1 μM concentration range. This observation questioned the interpretation of a low K_m being indicative of a high affinity for P. More research is required to fully elucidate the mechanism of uptake, and how it is influenced by culture conditions and excision treatments.

Uptake by both *Zea mays* and *Glycine max* at the highest pretreatment concentrations was similar to that of *E. nitens*, and uptake increased approximately 5-fold in both of those

species at low pretreatment concentrations. *Pinus radiata* had a higher uptake than *E. nitens* at each concentration, but uptake by *P. radiata* was less than that of both *Zea mays* and *Glycine max* at the lowest pretreatment concentrations.

Figure 9.1 - Comparison of rates of uptake by various species with different pretreatments (0 - 1 µM P in solution, for references to species other than *E. nitens*, see Table 2.5). Pretreatment in parentheses.



Sands and Smethurst (1995) showed that critical concentrations for growth could be calculated for relative-addition-rate (RAR) experiments, but not enough is known about the effects of relative-growth-rate on I_{max} for this approach to accurately calculate the critical concentration. Despite this drawback, the range of possible critical P concentrations predicted for *E. grandis* (0.45 - 2.64 µM) was at the lower end of values published for other species in solution culture (0.4 - 25 µM in Table 2.3). The I_{max} calculated for *E. grandis* roots (138 nmol/g FW/hour) in the RAR experiment of Kirschbaum *et al.* (1992) was well within

the range published for other species (eg. 31 – 1150 nmol/g FW/hour in Table 2.5), and similar to that found directly for *E. nitens* roots (43 nmol/g FW/hour).

Clarkson (1985) and Smart and Bloom (1993) suggested that growth would not be limited by N if the concentration of N at the root surface approximated the K_m of the high-affinity uptake system. This hypothesis was also supported for P in the current experiments with *E. nitens*. The K_m of the high affinity transport system of *E. nitens* roots was 0.37 μM, and the critical concentration found for *E. grandis* was 0.45 μM when decreasing I_{max} with solution P concentration was accounted for in the Sands and Smethurst (1995) model. The value of K_m was also similar to the optimum concentration of P in soil solution for *E. nitens* in pot experiments (0.19 - 0.58 μM), and the critical $CaCl_2$ P concentration for *E. nitens* and *E. globulus* in the field (0.50 μM).

9.2 Potential effect of mycorrhizae

The K_m of the high affinity transport system of *E. nitens* roots (0.37 μM) was lower than published values for the K_m of germ tubes of a vesicular-arbuscular mycorrhizal fungus (1.8 - 3.1 μM, Thomson *et al.* 1990), and 3 strains of ectomycorrhizal fungi (1.5 - 13.1 μM, Jongbloed *et al.* 1992). Although species variation occurs between fungi (Jongbloed *et al.* 1992), the K_m of *E. nitens* roots was lower than that of the published mycorrhizal fungi above. This comparison supported the theory that mycorrhizae allow better soil exploration but are not more effective at absorbing P from solution (eg. Bolan 1991). Hence, the critical concentration of P in soil solution is likely to be relatively independent of mycorrhizal association. Supporting this hypothesis, mycorrhizal infection had little effect on P fertilizer required for maximal growth of eucalypt (Bougher *et al.* 1990), and soybean (Plenchette and Morel 1996) seedlings. In both of those studies, mycorrhizal infection increased growth only at concentrations below optimal.

The mechanism of P transfer from ectomycorrhizal fungi to roots is uncertain, but if the

plasma membrane of root cells has a higher affinity for P than the hyphal membrane, P could be transferred by a source-sink relationship, with roots better able to absorb P at the root surface than hyphae.

9.3 Traditional P indicators

Soil P analyses typically extract all of the solution P and varying proportions of the quantity (or labile) form of P. Separating available soil P into quantity and intensity pools is a crude simplification of the highly heterogeneous soil system, but is a useful concept for discussion of plant P availability. The absolute amount of P in the solution phase (ie. P intensity) is generally very small compared with the quantity of P taken up by plants. For example, calculations in Chapter 6 showed that the amount of P taken up by plants was greater than 100-fold that present in solution, even in relatively short-duration (60 - 80 d) pot experiments. Similar observations have led many researchers (eg. Bray and Kurtz 1945, Colwell 1963, Dalal and Hallsworth 1976, Fixen and Grove 1990) to use chemical extractants in an attempt to mimic the amount of P available (in both the quantity and intensity pools) to plant roots. However, the quantity of P extracted by these analyses is usually only empirically correlated with plant response, because they extract different proportions of the labile P in each soil type, and P supply to roots is a dynamic process, involving interactions between soil P, solution P and plant roots. Barraclough (1989) related Olsen P to solution concentration in one soil type, and predicted the Olsen P required at the start of the growing season to ensure that the critical concentration of solution P was maintained at the root surface for growth of rape during the season. Although Barraclough (1989) mechanistically linked Olsen P and plant growth, soil P quantity analyses would need recalibration for different soil and crop types.

Labile P in forest soils is often extremely low, with most of the P occluded in organic matter, so researchers have turned to extractable organic P indices to describe the P status of forest soils (McLaughlin, 1996). However, in many soils (such as the Ferrosols investigated in this

study), a large proportion of the available P is sorbed to Fe and Al oxide and hydroxide minerals. Major disruptions to nutrient cycling occur during clearing and plantation establishment, with inorganic P becoming the main form of labile P (Adams 1992, Romanya *et al.* 1994), so the ability of organic P indices to predict response in new plantations is uncertain. The potential for inorganic P indices to predict P deficiency in eucalypts was investigated in the current study, and useful empirical correlations were found between P analyses and growth of *E. nitens* and *E. globulus* (Chapter 7).

All Colwell P test values in fertilized treatments of the second pot experiment had a decreasing trend with time, although only one of the regressions was significant. Hence values of P analyses from fertilized soils are not directly comparable with those from unfertilized soils until equilibrium is reached. Thus using recently fertilized soil in pot experiments is likely to lead to overestimation of critical levels of quantity indicators. This was exemplified by the difference between Colwell P values in fertilized and unfertilized soils. The fertilized clay loam 2 in Chapter 6 had a Colwell P range of 10 - 600 μg/g, but the same soil type (ie. Brown Ferrosol) without P fertilizer in Chapter 7 had a range of Colwell P between 2 and 60 μg/g. Equilibrium may take several years to be reached, as it was not reached by Barrow and Shaw (1975) in a fertilized loamy sand, even after the equivalent of 1000 days at 25°C.

$CaCl_2$ P was the best indicator of response to soil P status in both pot and field experiments, but optimal growth in the field was attained at a higher $CaCl_2$ P concentration (0.50 μM) than in glasshouse experiments (0.20 μM). Differences in soil water content between the glasshouse and field situations may explain the contrasting critical $CaCl_2$ P concentrations, because soil water content directly influences the effective diffusion of a nutrient in soil (Equation 2.4). Pots in the glasshouse experiments were watered frequently (3 times weekly), while soil in the field may have been drier on average. The duration of the experiment may also have caused an increased requirement for $CaCl_2$ P, as the concentration at the root

surface probably decreased over time in the field. Temperature may also have affected the critical concentrations. Higher temperatures in the glasshouse may have led to higher influx rates across the root plasma-membrane, resulting in the lower observed critical concentration. The latter explanation is less likely to explain differences between field and pot experiments, because although the field experiments covered a wide climatic range, response to P fertilizer was well correlated with $CaCl_2$ extractable P, and did not appear to be influenced by climatic regime.

The Colwell analysis extracted more P from soil than other analyses, suggesting that it was a good indicator of the quantity of P on the labile phase. $CaCl_2$ P was a useful indicator of solution P concentration, but it was influenced to a minor extent by buffer power between soil types, caused by dilution during extraction. The highly P-sorbing soil type in the first pot experiment (ie. the Brown Ferrosol) had close-to a 1:1 relationship between $CaCl_2$ P and solution P, but the sand in the second pot experiment (ie. the lowest P sorbing soil) had a 1:3 relationship between $CaCl_2$ P and solution P (ie. the concentration of P in the $CaCl_2$ extract was 1/3 of that in the soil solution).

The range of equilibrium P buffer capacity (EK_D) in soils from the 24 *Eucalyptus* nutrition experiments in southern Australia (6.64 - 48 475 mL/g) was wider than the range in 26 Queensland agricultural soils (159-15 702 mL/g) investigated by Moody *et al.* (1988), indicating a wide range of phosphate characteristics of the soils. The Colwell P range of the 24 plantation soils (2-63 ppm, median 9.2 ppm) was narrower than the range in the 26 Queensland soils (1-300 ppm, median 24.5 ppm). Hence, the amounts of P extracted by soil P quantity analyses were generally lower in the plantation than agricultural soils.

The finding that eucalypt growth was better correlated with soil P intensity than quantity suggested that solution P replenishment from the labile phase and transport to root surfaces occurred at an equal or faster rate than uptake by roots in pot and field experiments. It could be hypothesised that soil P quantity would be better correlated with growth in situations

where solution P is insufficient and uptake rate exceeds the rate of supply of P from the labile phase, for example after extended periods (years) of P uptake, or where a high level of competition exists between roots for the same P source.

Published reports in which growth was well correlated with P quantity indicators have often been pot experiments, where high levels of root competition would be expected (eg. Dalal and Hallsworth 1976, Holford and Mattingly 1976, Kadeba and Boyle 1978, Holford 1983), or conducted over extended periods of time (eg. in a *Pinus* plantation, Ballard and Pritchett 1975). High correlations are also found between wheat growth response and soil P quantity (Holford and Cullis 1985, Holford *et al.* 1985). High levels of competition between wheat roots in the field would be expected because they are planted at very close spacings, and their roots occupy the same niches in soil. Under circumstances of high competition, it is not surprising that release of P from the solid phase becomes the limiting factor to growth. In situations where competition for P was minimal (eg. where new roots, mycorrhizal hyphae or root hairs were growing in undepleted soils), and plant uptake was the limiting factor, then soil P intensity should be well correlated with P uptake. This has been found to be the case in short-duration pot experiments with maize (Moody *et al.* 1988), and sorghum-sudangrass (Soltanpour *et al.* 1974). Some crops such as soybeans (Moody *et al.* 1983) and subterranean clover (Gunary and Sutton 1967, Dear *et al.* 1992) respond well to P intensity over the duration of the growing season, suggesting that the rate of P uptake from soil solution was slower than the rate of replenishment of solution P to its equilibrium level. Both soybeans and subterranean clover have mycorrhizal associations (eg. Smith *et al.* 1981) which might minimize competition between roots via increased exploration of the soil, including pores too small for root penetration. Smaller soil pores would also have greater nutrient availability, because of higher water content (Equation 2.4).

Studies with *Pinus* species have shown a marked dependence on soil P quantity (Ballard and Pritchett 1975, Skinner *et al.* 1991). The reason for dependence on soil P quantity may be

root physiology, because solution P requirements of *Pinus* sp. appear to be high compared with the eucalypts in this study. For example, the uptake kinetic parameters of the high affinity transport system of *Pinus radiata* roots described a higher uptake rate than *E. nitens* in this experiment (Figure 9.1). Skinner and Attiwill (1981) found that the optimum concentration of P in solution for *Pinus radiata* was 3-6 μM, Tiarks (1982) found an optimum soil solution P concentration of 6.5 μM for *Pinus taeda.* Hence, P supply may limit growth in *Pinus* species, because the uptake system removes P from soil at a faster rate than it is replenished. Although *E. nitens* roots had a low uptake rate compared with other species (Figure 9.1), they may have mechanisms to increase the natural ability of the soil to supply P.

9.4 Modelling P supply and uptake to predict P deficiency

The Barber-Cushman and modified Smethurst-Comerford models predicted a similar level of uptake in soils with a wide range of P buffer powers (Chapter 8 and Chapter 9), indicating that both methods of calculation of P at the root surface provided similar results. The advantages of the Smethurst-Comerford model were that it accounted for changing P buffer power with concentration of P in solution, and it permitted variable root length density during the simulation.

Output of nutrient supply and uptake models (ie. predicted concentration of P at the root surface, and predicted uptake) was well correlated with observed growth and P uptake for *Zea mays* in a range of soil types, and with growth of *Eucalyptus nitens* within each soil type in pot experiments, but model predictions were not better correlated with growth than measures of soil P intensity for both species. The dependence of *Zea mays* on P intensity (Chapter 8) may have been due to the short duration of the experiment (25 d), because growth of *Zea mays* in longer-term field experiments on Ferrosols was better correlated with Colwell P (an indicator of soil P quantity) than with P intensity or capacity indicators (Moody *et al.* 1997). The modelling approach may be useful in the longer term for *Zea mays* over a wide range of soil types, because it integrates P supply and uptake theory to calculate P intensity at

the root surface.

Standard P availability indices have an asymptotic relationship with P uptake, where the level of the asymptote is dependent on the growth limiting factor. A considerable advantage of the modelling approach for prediction of P deficiency in maize was the linear relationship that was derived between observed uptake and both predicted concentration at the root surface, and predicted uptake (Mendham *et al.* 1997). Predicted solution concentrations of P at the root surface were all below the value of K_m, so the linearity of the relationship was explained by the near-linear form of the Michaelis-Menten uptake curve between C_{min} and K_m. Such a relationship would be useful for predicting P uptake by plants growing in locations with different growth potentials. For example, maximum growth may be lower at a colder than a warmer site, so plants at the colder site would require less P. Crop growth models are available (eg. Jones and Kiniry 1986, for maize) that predict maximum plant growth, based on environmental variables such as radiation and temperature. Nutrient supply and uptake models (such as the Smethurst-Comerford model) could predict solution P required in a particular soil-plant system to ensure that P is not limiting growth in that system.

Phosphorus supply and uptake theory used by mechanistic models such as the Barber-Cushman and Smethurst-Comerford models is not yet advanced enough to accurately predict P uptake by *E. nitens*. Published studies which have used the Barber-Cushman model to predict uptake by other species also show that predicted uptake is often less than observed, especially at low solution concentrations. For example, P uptake predicted by the Barber-Cushman model was only 20% of observed uptake at concentrations less than 30 µM for *Zea mays* (Lu and Miller 1994), and 30-35% of observed uptake in *Glycine max* at low solution P (Silberbush and Barber 1984). Even at high solution concentrations (190 µM), predicted uptake was 89% of observed uptake in *Pinus taeda* (Kelly *et al.* 1992). The Smethurst-Comerford model also underpredicted P uptake by *Pinus elliottii* at low P concentrations (24-30% of observed, Smethurst and Comerford 1993b). Hence, theory of P supply and uptake

used by these models has been inadequate in a number of published studies, as well as for eucalypts in this study.

The concentration of P required at the root surface for observed uptake by *E. nitens* was similar to that measured in bulk soil solution in pot experiments (calculated from the measured quantity of roots and uptake kinetic parameters, Section 9.3.1), indicating that the concentration at the root surface was higher than that predicted by current theory of P supply and uptake. The ratio of observed:predicted uptake was influenced by P buffer power, so accounting for buffer power could lead to useful correlations with response to P fertilizer. However, P buffer power has a relatively minor role in influencing uptake (as indicated by high correlations between response and $CaCl_2$ P), so correlations between buffer power and model output were probably present because buffer power was initially factored into the model.

Gaps in knowledge of P supply and uptake principles which were found to hinder useful application of mechanistic models in this study include (a) the effect of external and internal concentrations on the values of I_{max}, (b) effect of time on P sorption, (c) rhizosphere chemistry, and (d) root growth. Uptake parameters, particularly I_{max}, change with nutrient status (Jungk *et al.* 1990, Dunlop *et al.* 1997), root age and root type (Bowen 1969), and microbial complement (Temple-Smith and Menary 1974), which affects uptake predicted by mechanistic models. It was shown that changing the I_{max} had a large effect on predicted P uptake by *E. nitens* seedlings when P buffer capacity was assumed to be infinite, but not at lower values of P buffering. Increasing I_{max} caused a reduction in predicted concentration of P at the root surface, so the effect on uptake was minimal.

Buffer power at the root surface may be higher than that measured during a short-term sorption analysis, because longer-term sorption increases buffer power over time (Barrow and Shaw, 1975), and root processes that increase the availability of P (such as rhizosphere acidification, and exudation of phosphatase enzymes/organic acids) would also increase

effective buffer power.

Predicted concentrations of P at *E. nitens* root surfaces were much lower than was feasible for the observed uptake. Predicted uptake was similar to that observed when infinite P buffering capacity was assumed (ie. the root surface concentration was the same as that in bulk soil solution), indicating that P concentration at root surfaces was similar to that in bulk soil solution. Hence, the roots appeared to increase P availability in the rhizosphere, compared with the amount predicted by models of P supply and uptake. Mechanisms to account for this apparent P release in the rhizosphere include acidification and exudation of phosphatase enzymes. Acidification can occur via extrusion of protons (in exchange for nutrient cations), or organic acids. Acidification in soils below neutral pH effects release of P (Grinsted *et al.* 1982, Saleque and Kirk 1995), probably by increasing competition of other anions (eg. sulfate) for sorption sites at lower pH (Marsh *et al.* 1992, Geelhoed *et al.* 1997). Conjugate anions of organic acids (such as oxalate and malate) which are exuded by roots may also substitute for P sorbed to the solid phase (Comerford and Skinner 1989), but only in the short term (eg. months, Afif *et al.* 1995). Phosphatase release by plant roots mediates conversion of organic phosphate into inorganic P which can be taken up by plant roots (Tarafdar and Claassen 1988). Increasing solution concentration of P by 10-fold had a greater effect on predicted uptake than any of the other parameters investigated in the error analysis (Chapter 9), and Silberbush and Barber (1983) also found that concentration of P in solution had the second highest influence (after root growth) on predicted uptake, even though they only increased the level of P in solution by a factor of 2.

Root growth is another aspect of plant uptake that is poorly accounted for. Detailed measurement involves painstaking sampling techniques which must be conducted several times during the growth of the plant in order to estimate both quantity of roots and form of root growth over time. The effects of soil structure, nutrient status, or water content on root growth have been quantified in a number of studies (Goss *et al.* 1992, Eissenstat and Van

Rees 1994), and soil strength (due to compaction) has been shown to decrease root length and increase diameter of *E. nitens* roots (Misra and Gibbons 1996). However, integrating these effects into a model of root growth would require information on soil heterogeneity, which would be time-consuming to collect, and site specific. Hence, practical use of such information in nutrient uptake models is unlikely.

The modelling approach showed potential, but more knowledge is required about the P supply and uptake system, especially chemistry in the rhizosphere of eucalypt roots, before such an approach would be feasible for prediction of response to P fertilizer in eucalypt plantations.

9.5 Predicting P deficiency in eucalypt plantations

The level of P required in soil solution (0.19 - 0.58 μM) for maximum growth of eucalypts is significantly lower than that required by most introduced agricultural (including pasture) species (see Table 2.4). Hence, plantations on ex-agricultural (or ex-pasture) sites should not require P fertilization if those sites have been fertilized to ensure survival and economic growth of the agricultural species. This expectation was partly demonstrated in Chapter 7, where the ex-pasture site had the highest P test levels of all of the quantity indices, and third highest $CaCl_2$ P. Minimal response to P fertilizer occurred at that site.

For soil tests on sites of lower natural fertility (eg. ex-*Pinus* plantations, and ex-native forest), the location of soil sampling needs special consideration. $CaCl_2$ P analysed from inter-mound soil samples (0-10 cm) was best correlated with growth at 10 Tasmanian sites, and that soil sample was best correlated with $CaCl_2$ P in soil from undisturbed B-horizon soil (eg. 20-30 cm), suggesting that surface layers of soil have been lost from the inter-mound.

It was assumed that *E. nitens* and *E. globulus* were similar in their response to soil P, but the evidence was equivocal. No significant difference was found between *E. nitens* and *E. globulus* in the third pot experiment (Chapter 6). The reason for the lack of difference may

have been that the two species were similar in their response to P, or that variability was too high to fully characterise the differences between them. Variability was high, but *E. globulus* reached maximum growth at a lower $CaCl_2$ P concentration than *E. nitens*. Similarly, the 3 outlying sites on the $CaCl_2$ relationship with relative yield in the field experiments (Chapter 7) were also *E. globulus* (although the other 5 *E. globulus* sites were well described by the same linear trend as *E. nitens* in that relationship). Hence, *E. nitens* and *E. globulus* were similar (compared to other species), but they may not have been exactly the same in their response to soil P. Further research would be required to fully elucidate the difference (if any) between the two species.

Significant correlations were found between P quantity indices and response to P fertilizer in *E. nitens* and *E. globulus* plantations. Critical values of such analyses were similar to those of agricultural crops, but there was high variability. Such analyses would not be useful for prediction of response to P fertilizer, unless very high values were used as the critical levels. Hence these P analyses would not discriminate between sites with enough accuracy for a confident prediction of response to P fertilizer. Soil P sorption capacity was not the main factor influencing variation in quantity analyses, because multiple regressions with indicators of P buffer capacity and quantity were not more significant than with quantity analyses alone. The explanation was probably that different soil components react differently to the Colwell extract in each soil.

Fertilizer rates were not considered in this project, as the aim was to identify sites that would respond to P fertilizer, rather than define the quantity of fertilizer required to attain maximum growth. The relationship between $CaCl_2$ P and growth was well correlated in both pot and field experiments, so it would probably be a useful indicator of the rates of P required for maximum growth. Hence, an effective fertilization régime could probably be developed, but would be specific to soil type. For example, soils with high P sorption would require more P fertilizer than soils with low P sorption (Barrow 1989). Sorption curves are a useful tool for

determination of fertilizer requirement in agricultural crops (Fox 1980), as they give an indication of the quantity of P fertilizer required to increase the concentration of P in soil solution to the critical level for plant growth. This approach would not be as practical for plantation forestry, because P fertilizer is usually applied at planting individually to each tree in a concentrated dose (ie. in a small volume of soil). This form of application has advantages over broadcasting in the forestry situation, because competing vegetation has less access to the fertilizer, and less P is sorbed to the solid phase of the soil. The relationship between $CaCl_2$ P and rates of P fertilizer (spot applied) would probably need to be calibrated for each soil type.

In conclusion, $CaCl_2$ P was predominantly influenced by the solution P concentration, with a close to 1:1 relationship in highly buffered soils, and a decreasing solution:$CaCl_2$ P ratio with decreasing buffer power. However, buffer power had only a minor influence on $CaCl_2$ P concentration compared with Colwell P. Holford (1991, 1997) suggested that a P analysis should incorporate both P capacity and P intensity components for it to be successful. In the case of *E. nitens* and *E. globulus*, $CaCl_2$ P gave the best integration of soil P quantity and intensity that influenced P uptake by temperate eucalypts in both field and glasshouse experiments. $CaCl_2$ P was well correlated with response to P fertilizer at 21 sites from a wide geographic and climatic range, including Tasmania, Victoria, New South Wales, and West Australia. The $CaCl_2$ P analysis could be utilized for site-specific management of new eucalypt plantations, with sites above 0.5 μM $CaCl_2$ P not requiring P fertilizer.

10. Conclusions

Several soil P analyses were assessed for their ability to predict response to P fertilizer in *Eucalyptus nitens* and *E. globulus* plantations. Major conclusions that can be drawn from this study are presented below.

10.1 Forms of P in soil

Inorganic forms of P were the focus, because it was considered that inorganic processes probably dominate P availability during the initial stages of plantation establishment. Of the soil P quantity indicators investigated (Colwell, Bray No. 2 and Acid-extractable P), Colwell extracted the most P, and was highly influenced by buffer power. Concentrations of $CaCl_2$ P were mainly influenced by solution P, but low P-sorbing soils also had lower concentrations of $CaCl_2$ P than solution P, probably due to a dilution effect. Useful ranges of these analyses were obtained, but test results were generally lower than those previously reported for agricultural soils.

10.2 Indicators of P availability

Indicators of soil P quantity (Colwell, Bray No. 2 and Acid-extractable P) were significantly correlated with response to P fertilizer, and had critical concentrations (ie. the concentration at 90% of predicted maximum growth) in the field of 21 - 71 µg/g for Colwell analysis, 5 - 24 µg/g for the Bray No. 2 analysis, and 2 -17 µg/g for Acid extractable P. Wide ranges of those indices were present, due to inter-site variability. Although quantity based analyses were significantly correlated with response to P fertilizer, such analyses would not be appropriate for use in the field situation because of the widely different critical concentrations which were calculated. $CaCl_2$ P was more highly correlated with response to P fertilizer in both pot experiments, and at 1 year of age in the field. The critical concentration for 90% of maximum growth was 0.20 µM in glasshouse experiments, and 0.50 µM in the field.

10.3 Relations between K_m and maximum growth

The measured K_m for P uptake by *E. nitens* roots (0.37 μM) was similar to the critical concentration calculated for *E. grandis* (0.45 - 2.64 μM), similar to the critical bulk soil solution P concentration found for *E. nitens* in pot experiments (0.2 - 0.6 μM), and similar to the critical $CaCl_2$ P concentration found for growth of *E. nitens* and *E. globulus* in the field (0.50 μM). All critical solution and $CaCl_2$ P concentrations were at the lower end of the range of critical concentrations published in the literature, supporting the hypothesis that eucalypts have evolved mechanisms to utilize the low levels of available P in most Australian soils (Handreck 1997).

10.4 Pot experiments vs field experiments

Pot experiments were useful for identifying the relative importance of each of the analyses, but critical concentrations for quantity indicators that were derived from pot experiments were not useful, because the fertilized soils were not in equilibrium. Hence, much more Colwell P was extracted per unit of solution P than with unfertilized soils. Results from field experiments were more applicable because those experiments covered a wider climatic range and a longer time scale. Only data from trees of 1 year of age in the field was assessed, because responses to P fertilization in the first year are generally maintained (Schönau and Herbert 1989).

10.5 Modelling approach - problems and future research

Phosphorus response of eucalypts was more closely related to the concentration of P in bulk soil solution, rather than that predicted at the root surface. For such models to be useful for predicting P deficiency in eucalypt plantations, more knowledge is required about interactions between soil, roots, and mycorrhizae in the rhizosphere, and about dynamic changes in root growth and P uptake mechanisms in soil.

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